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The bare-nosed wombat and its pathogen, *Sarcoptes scabiei*

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Declarations by the author

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Abstract

Thesis abstract

An unintended consequence of the global movement of humans and their domestic animals has, and continues to be, the introduction of pathogens to naïve host species. Such pathogen invasion events have consequences for wildlife ranging from benign to catastrophic. Yet, the specific impacts to affected species – from individuals to populations – and the capacity to mitigate these are often poorly understood. This is perhaps best illustrated in mammals by the parasitic mite, *Sarcoptes scabiei* (causative agent of sarcoptic mange): among the most successful of known pathogens to benefit from anthropogenic globalisation. The mite now occurs on all continents (except Antarctica) and has been documented infecting >100 mammal species, yet the impacts of this parasite remain enigmatic for most wildlife species it infects.

In Australia, evidence indicates *S. scabiei* was introduced by European settlers. Over the last 200 years, *S. scabiei* has been documented in several native and non-native Australian mammals, with most striking impacts observed in the bare-nosed (common) wombat, *Vombatus ursinus*. Though sarcoptic mange causes conservation, ethical, and welfare concerns to *V. ursinus*, considerable knowledge gaps have persisted about the effects of the parasite, including the impacts of infection on individuals and at population scales, as well as the capacity to mitigate infection. Furthermore, there is a dearth of fundamental knowledge about the *V. ursinus* host, despite being a perceived "common" species. The aims of this thesis were therefore four-fold: (i) to identify behavioural and physiological impacts of *S. scabiei* presence at the individual level, (ii) to quantify the population-scale impact of a sarcoptic mange outbreak in a bare-nosed wombat population, (iii) to evaluate the efficacy of the current suggested treatment protocol administered at a population-scale, and (iv) to understand the genetic structure of *V. ursinus* across its entire range (encompassing three subspecies).

To understand the impacts of sarcoptic mange at the individual level, I focused on physiological and behavioural changes observed in mange-infected bare-nosed wombats. Specifically, I investigated the effect of sarcoptic mange infection on heat loss, field metabolic rate, resting and foraging behaviour, and fatty acid composition (Chapter 2). I used methods in thermal imagery, doubly labelled water, activity loggers, and fat composition profiles to reveal that as mange severity increased in wombats, (i) heat lost to the environment increased, (ii) field

metabolic rates increased, (iii) total time spent foraging decreased while total time spent inactive increased, and (iv) fatty acid composition changed in adipose tissue. I concluded that the compounding effects of physiological and behavioural changes left infected wombats unable to meet the energetic demands of sarcoptic mange disease.

The pattern of disease spread and impact of an outbreak on bare-nosed wombat abundance was assessed at Narawntapu National Park (north-central Tasmania) during a mange epizootic. I used seven years of population data and four years of disease severity data in combination with piece-wise linear regressions and geographic heat maps to understand the consequence of mange outbreak in a semi-isolated wombat population. I found that disease spread through Narawntapu National Park spatiotemporally, in a wave-like pattern from east to west, and caused a >94% decline in wombat abundance from 2010-2016 (Chapter 3). Furthermore, I suggested that sarcoptic mange outbreaks can result in localised extirpation in semi-isolated populations.

During the outbreak event at Narawntapu National Park, I attempted a population scale treatment regime based on the current suggested treatment protocols (Chapter 4). I administered treatment to >200 burrows for 12 consecutive weeks and performed disease severity surveys for 18 months to quantify the efficacy of the treatment regime. I found that current treatment methods provided temporary protection, but were not sufficient to eradicate mange from the park, and reinfection occurred shortly after treatment ceased. Using a novel, purpose-built, state-based model fitted to empirical data from the treatment trial, I explored practical changes to current treatment methods that may influence treatment outcomes. I found that treatment application success was low using current techniques, and that a longer lasting treatment may facilitate capacity for future population scale disease control.

Finally, I sought to develop a baseline understanding of *V. ursinus* genetic structure across its range, as well as address the currently accepted subspecific claims (mainland *V. u. hirsutus*, Flinders Island *V. u. ursinus*, Tasmania *V. u. tasmaniensis*). Using genome-wide single nucleotide polymorphisms, I identified (i) three genetically distinct groups, consistent with current subspecies classifications, (ii) a second population of the Vulnerable *V. u. ursinus* on Maria Island (an offshore island of Tasmania), and (iii) isolation by distance across the

Tasmanian range (Chapter 5). I concluded that the three subspecies may better be managed as separate units.

The work presented in this thesis has contributed to a greater understanding of: (i) the impacts of sarcoptic mange, at the individual and population level, in bare-nosed wombats, (ii) the efficacy of current treatment methodologies and potential improvements to future management regimes, and (iii) the baseline genetic structure of *V. ursinus*, identifying three genetically distinct subspecies that may warrant separate management action. The contributions I have made to *V. ursinus* and *S. scabiei* research will help to inform future disease management efforts for this species and provides insight to management of evolutionary significant units within the *V. ursinus* range. Moreover, this thesis contributes to the broader understanding of an invasive, globally significant, and environmentally transmitted pathogen, with conservation implications for other impacted species and similarly transmitted invasive pathogens impacting wildlife.

Chapter 1

General Introduction

Chapter 1.0 – General Introduction

1.1 Impact of wildlife disease

Many important diseases of wildlife are invasive (Tompkins *et al.* 2015). Pathogens are often unintentionally transported via anthropogenic movement (termed 'pathogen pollution'), and can pose substantial threats to naïve hosts in their expanded ranges (Daszak *et al.* 2000). However, prior to 2000, wildlife diseases were often of minimal research priority, save for those that impacted human and domestic animal health, or human interest (e.g., diseases affecting game species) (Cunningham *et al.* 2017). In the wake of recent invasive pathogens driving wildlife population declines, the previously accepted notion that disease alone could not be an agent for extinction has been challenged (De Castro and Bolker 2005), shifting the focus of much research over the last two decades toward understanding the impacts of pathogens on wildlife.

Invasive pathogens can have variable impacts within and among wildlife species, and there can be diverse underlying mechanisms associated with impacts. For example, there may be differences in pathogen invasion dynamics, as well as variability in the susceptibility, prevalence, and morbidity among individual hosts and species. The spectrum of population impacts caused by invasive pathogens on wildlife species can therefore range from benign, to catastrophic declines, to extinction events. Further, establishment of invasive pathogens which impact new host species can suppress host abundance long after invasion and cause sporadic outbreak events. For example, two of the most successful invasive pathogens of wild mammals, plague (causative agent *Yersinia pestis*) and sarcoptic mange (causative agent *Sarcoptes scabiei*), which affect >200 and >100 species (respectively), can be both enzootic and epizootic within and across host species (Biggins and Kosoy 2001, Pence and Ueckermann 2002).

Diverse effects of invasive pathogens on new host species have consequences for understanding individual and population scale impacts, as well as for informing disease control. For example, some invasive pathogens of wildlife cause significant individual pathology, but only cause population level impacts under specific conditions. A specific example includes anthrax, causal agent *Bacillus anthracis*, which impacts mammals globally. Prevalence can

remain rare or low in hosts (Hugh-Jones and de Vos 2002), but given the right conditions, can lead to epizootic events (Muoria *et al.* 2007, Blackburn and Goodin 2013). Therefore, comprehending the individual and population level impacts of invasive pathogens can inform the appropriateness and outcomes of disease control attempts. This is important because pathogen control remains one of the most challenging aspects of disease ecology (Joseph *et al.* 2013). The most effective management strategy may depend on several factors, including the spatiotemporal pattern by which a disease spreads through a population, the transmission pathways, survival of the pathogen off the host, and changes exhibited by the host because of infection.

1.2 Introduction to the "itch" mite, *Sarcoptes scabiei*

Among the most widespread of wildlife pathogens is *Sarcoptes scabiei*, the microscopic, burrowing, parasitic mite responsible for the skin disease sarcoptic mange (Bornstein *et al.* 2001, Pence and Ueckermann 2002). *Sarcoptes scabiei* is a parasite of global importance, present on all continents (except Antarctica) and infecting more than 100 mammal species (Pence and Ueckermann 2002). It is likely that *S. scabiei* has human origins (scabies), and spilled-over into domestic animals, and subsequently into wildlife species (sarcoptic mange) (Bornstein *et al.* 2001).

Despite the wide array of hosts, *S. scabiei* has relatively conserved pathology across species. Generally, the host exhibits a hypersensitivity response to the mite's presence, inducing the following clinical signs: intense pruritis, alopecia, skin lesions, skin thickening, skin discoloration and inflammation (Bornstein *et al.* 2001, Pence and Ueckermann 2002). The severity of these responses is determined by infestation intensity. Mange can present in two forms: ordinary (10-15 mites per host) and advanced (also called 'crusted' or 'Norwegian scabies,' thousands to millions of mites per host) (Walton *et al.* 2010, Arlian and Morgan 2017). Crusted mange results in a highly infectious host with higher morbidity (hyperkeratosis), and a longer lasting and refractory infection. Variability in host immune response contributes to the disease form exhibited, with immunosuppressed hosts more likely to exhibit crusted mange (Roberts *et al.* 2005, Malik *et al.* 2006). Severe infestations often result in secondary infections (Walton and Currie 2007), and in extreme cases, mortality. Crusted mange is commonly observed among

wildlife hosts, and has been documented in several species including red fox (*Vulpes vulpes*), coyotes (*Canis latrans*), lynx (*Lynx lynx*), ibex (*Capra ibex*), and wombats (*Vombatus ursinus*, *Lasiorhinus latifrons*) (Pence and Ueckermann 2002).

1.3 Impact of mange epizootics in wildlife

The effects of sarcoptic mange vary among hosts and geographic location, with observations ranging from a few, isolated incidents to epizootic events. Epizootic events can have varying population consequences, both within and between species. In some instances, populations recover and return to pre-outbreak densities, while others are driven to local extirpation (Smith *et al.* 2009). Risk of localised extinction is highest in small, isolated, or naïve populations.

There have been two documented outbreak events that have led to localised extirpation of wildlife populations: one in red fox (*V. vulpes*) and one in Spanish ibex (*Capra pyrenaica*). In the case of the red fox, *S. scabiei* was introduced into an isolated, island population that was likely naïve to the pathogen. Following the mange outbreak, foxes were extirpated from the island (Henriksen *et al.* 1993, Pence and Ueckermann 2002). In the second case, *S. scabiei* infected a Spanish ibex population in epizootic waves. The first epizootic outbreak created fragmented populations across the local range, and the second epizootic caused 100% mortality in at least one of these isolated populations (León-Vizcaíno *et al.* 1999). These events highlight the impact of mange epizootics on wildlife populations, in cases of both naïve and previously exposed hosts.

1.4 *Sarcoptes scabiei* in Australia: an invasive pathogen of wombats

It is well-supported that *S. scabiei* was introduced to Australia by European settlers and their domestic animals, and subsequently spilled-over into wildlife populations (Fraser *et al.* 2018a). The mite infects several native and non-native hosts, including koala (*Phasocolarctos cinereus*) (Obendorf 1983), southern brown bandicoot (*Isoodon obesulus*) (Wicks *et al.* 2007), agile wallaby (*Macropus agilis*) (McLelland and Youl 2005), swamp wallaby (*Wallabia bicolor*) (Holz *et al.* 2011), dingo (*Canis lupus dingo*) (Thomson *et al.* 1992), ringtail possum (*Pseudocheirus*

peregrinus) (Skerratt 2005), and two species of wombat (southern hairy-nosed, *Lasiorhinus latifrons*; and bare-nosed [also known as common], *Vombatus ursinus*) (Skerratt 2005, Ruykys *et al.* 2009). Of the native hosts, bare-nosed wombats experience higher prevalence, severe morbidity, and mortality.

Sarcoptic mange is particularly widespread in the bare-nosed wombat, occurring throughout its range at varying prevalence (5%-20%) (Martin *et al.* 1998). Outbreaks occur sporadically in wombat populations and are associated with notable declines in abundance (Martin *et al.* 1998). Furthermore, anecdotal reports suggest that mange may have contributed to the localised extirpation of bare-nosed wombat populations in New South Wales (Gray 1937), and in South Australia in the 1990s, but no evidence exists conclusively linking these declines to disease outbreaks. Advanced, or crusted, mange is often observed in bare-nosed wombats and is associated with the clinical signs of alopecia, parakeratosis, and skin fissuring (Skerratt 2003b). In addition to clinical signs, *S. scabiei* infection is responsible for an array of physiological and behavioural changes. Physiological changes include reduced fat stores (Martin *et al.* 1998, Skerratt 1998, Ruykys *et al.* 2009), decreased reproductive function (Skerratt *et al.* 1999, Ruykys *et al.* 2009), and loss of body condition (Skerratt *et al.* 1999, Skerratt *et al.* 2004b, Ruykys *et al.* 2009). Behavioural changes include increased diurnal activity (Hartley and English 2005, Simpson *et al.* 2016), increased time spent outside of the burrow (Simpson *et al.* 2016), and re-allocation of time spent engaging in different activities (Simpson *et al.* 2016). These effects likely have compounding impacts that result in mortality of the host.

1.5 The bare-nosed or "common" wombat, *Vombatus ursinus*

Despite being a species of perceived abundance, the bare-nosed wombat has received relatively little research attention, and there is a dearth of information regarding its basic biology and ecology. Across the *V. ursinus* range, three geographically isolated subspecies have been recognised: south-eastern mainland (*V. u. hirsutus*), Flinders Island (Bass Strait) (*V. u. ursinus*), and Tasmanian (*V. u. tasmaniensis*). These subspecies have been assigned based on regional isolation and morphological differences. While *V. ursinus* is considered to be of Least Concern (Taggart *et al.* 2016a), within these regions, the subspecies are subjected to conservation threats, including disease and habitat loss, and all have experienced range

retractions since European settlement (McIlroy 1973, Triggs 1998). Further, sarcoptic mange poses a risk to the Tasmanian and Flinders Island subspecies, where populations are smaller and more isolated.

1.6 Challenges in controlling *S. scabiei* in *V. ursinus*

There is widespread public interest in controlling *S. scabiei* in Australia, owing to the overt pathology it causes. The individual – and population – scale impacts of sarcoptic mange on bare-nosed wombats have made it a disease of both animal welfare, and occasional local conservation, concern. Several pharmaceuticals have been tested (and deemed effective) in clearing the mite infestation from individual wombats: ivermectin and moxidectin (Skerratt 2003b, Skerratt *et al.* 2004b, Ruykys *et al.* 2013). However, treatment of the disease is complex. Generally, multiple, consecutive doses (6-12) are required to clear the infection and should be administered weekly, as the drug is excreted from the system within 5 days (Death *et al.* 2011). Treatment efforts are complicated by mite survival in the environment. Transmission of *S. scabiei* can be through both direct and indirect transmission. Indirect transmission generally occurs through contact with environmental reservoirs of *S. scabiei*, as the mite can survive off the host up to 19 days in cool temperatures and humid climactic conditions (Arlian and Morgan 2017). These conditions are similar to those maintained by the wombat burrow, and thus the burrow likely plays an important role in mite survival, host reinfection, and disease transmission (Old *et al.* 2018). While bare-nosed wombats are primarily solitary, they will share burrows asynchronously; thus, the extended survival of *S. scabiei* in the environment enables transmission via burrow sharing among otherwise segregated hosts. Thus, an effective treatment effort must (i) clear *S. scabiei* infection from host, and (ii) protect the host from environmental reinfection while pathogen reservoirs deplete. Anecdotal reports suggest that current suggested treatment methods can clear individual wombats of mange disease, however, the efficacy of methods have not been explored at a population scale or during outbreaks.

1.7 Research aims

The purpose of this thesis is to evaluate the impacts and control of sarcoptic mange in the bare-nosed wombat host, as well as better understand aspects of bare-nosed wombat ecology. To address these larger objectives, I present four core chapters, in the form of independent publishable units, bound by this general introduction and a general discussion (Chapter 6).

Understanding the consequence of disease on individual hosts can reveal compounding mechanisms that lead to host mortality and inform potential management efforts. In Chapter 2, I explore the physiological and behavioural impacts of sarcoptic mange on bare-nosed wombats (Martin *et al.* 2018b). I address knowledge gaps regarding the impact of sarcoptic mange on heat loss, field metabolic rates, foraging behaviour, and fat composition, and find that mange infection incites an energetic burden that the wombat host cannot compensate for through behavioural plasticity. These findings may be informative to management aimed at reducing the impact of outbreak events in the wombat host (e.g., potential for food supplementation), as well as other species impacted by mange disease.

The pattern by which disease spreads through a population and the impact of outbreaks on host abundance can have implications for spread prevention and population recovery. In Chapter 3, I quantify the impact of a sarcoptic mange outbreak on bare-nosed wombat abundance and document pattern of disease spread through a population (Martin *et al.* 2018a). Using seven years of population surveys and four years of disease severity surveys, I find that sarcoptic mange spread through a wombat population in a wave-like pattern, progressing spatiotemporally from east to west, resulting in >94% abundance decline. These results suggest that barriers (either to host movement or via inciting host immunity) may be employed to slow disease spread, and that outbreak situations can result in localised host extirpation, in some instances. Furthermore, patterns observed here may be informative to disease spread and consequence in other solitary hosts where *S. scabiei* is transmitted via an environmental reservoir.

Controlling disease in wildlife populations is of widespread interest but is difficult to implement. Specifically, control of *S. scabiei* in *V. ursinus* has been a matter of ethical concern, yet current treatment protocols have only been used at an individual scale. In Chapter 4, I assess the efficacy of current treatment methods in controlling mange disease at a population scale

during an epizootic event (Martin *et al*. in review). Further, I use the data from the field trial to parameterize a novel, state-based model to investigate plausible changes to current treatment methodologies which may increase success of future treatment regimes. These findings may be applied in the *S. scabiei* and *V. ursinus* system, as well as other host-disease systems where the pathogen is persistent in the environment.

While *V. ursinus* has experienced range retractions and is threatened on multiple fronts (e.g., disease and habitat loss), no work has been done to identify important or at-risk populations within and across subspecies. In Chapter 5, I explore the genetic structure of the bare-nosed wombat across its range and address current subspecies delineations (Martin *et al*. in review). Using genomic data, I identify three genetically distinct groups, consistent with current subspecific classifications. I also identify a second population of the Vulnerable Bass Strait subspecies and address the impact of fragmentation on population connectivity and health.

In my final chapter, I synthesize key results across my thesis, discuss management implications of this body of work, as well as broader applications beyond wombats and *S. scabiei*, and lastly future research directions.

Chapter 2

Chapter 2.0 – The cascading pathogenic consequences of Sarcoptes scabiei infection that manifest in host disease

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2.1 Abstract

Sarcoptic mange, caused by the parasitic mite *Sarcoptes scabiei*, causes a substantive burden of disease to humans, domestic animals, and wildlife, globally. There are many effects of *S. scabiei* infection, culminating in the disease which hosts suffer. However, major knowledge gaps remain on the pathogenic impacts of this infection. Here, we focus on the bare-nosed wombat host (*Vombatus ursinus*) to investigate the effects of mange on: (i) host heat loss and thermoregulation, (ii) field metabolic rates, (iii) foraging and resting behaviour across full circadian cycles, and (iv) fatty acid composition in host adipose, bone marrow, brain, and muscle tissues. Our findings indicate that mange infected *V. ursinus* lose more heat to the environment from alopecia affected body regions than healthy individuals. Additionally, mange infected individuals have higher metabolic rates in the wild. However, these metabolic demands are difficult to meet, since infected individuals spend less time foraging and more time inactive relative to their healthy counterparts, despite being outside of the burrow for longer. Lastly, mange infection results in altered fatty acid composition in adipose tissue, with increased amounts of omega-6 acids, and decreased amounts of omega-3 acids, a consequence of chronic cutaneous inflammation and inhibition of anti-inflammatory responses. These findings highlight the interactions of mange induced physiological and behavioural changes, and have implications for the treatment and rehabilitation of infected individuals.

Keywords: *Vombatus ursinus*, Sarcoptic mange; pathophysiology; metabolic rate, fatty acid composition, time budget

2.2 Introduction

The condition of 'disease' conferred upon hosts by infectious organisms is a manifestation of cascading pathogenic effects following infection, the summation of which can translate to effects on population, community and ecosystem scales. Yet, for many important wildlife diseases, the cascades underscoring disease manifestations remain poorly understood. This is particularly consequential where the host range of infectious organisms may be expanding, such as is often the case with emerging infectious diseases. Importantly, understanding the cascading consequences of infection can provide insights across existing and new host species for the development of strategies for treatment and rehabilitation of individuals, as well as the prevention and management of disease transmission.

Sarcoptes scabiei is a globally widespread parasitic mite and the aetiological agent of sarcoptic mange disease in humans (scabies), domestic and wild animals (Bornstein *et al.* 2001, Pence and Ueckermann 2002). Mange is among the 30 most common human infectious diseases with an estimated 300 million cases annually, and was recently listed by the World Health Organisation as a "Neglected Tropical Disease" (Walton and Currie 2007, WHO 2017). This parasite causes a significant burden of disease and has been documented to infect more than 104 mammal species, spanning seven families (Bornstein *et al.* 2001, Pence and Ueckermann 2002). Mange is known to be particularly severe to some host species, including *Vulpes vulpes* (red fox), *Capra pyrenaica* (Spanish ibex) and *Vombatus ursinus* (bare-nosed wombat) (Pérez *et al.* 2002, Skerratt 2005, Soulsbury *et al.* 2007). The global host range of *S. scabiei* continues to expand, and represents a significant emerging infectious disease (Tompkins *et al.* 2015).

Infection by the *S. scabiei* mite confers a diverse array of impacts on hosts. Clinical signs of mange are often observed four (or more) weeks post exposure, when the mites have established a population on the host (Arlian and Morgan 2017). During the early stages of infestation (the first few weeks), mites are able to suppress the host immune response. However, as mite densities increase, the host begins to exhibit an initial hypersensitivity reaction resulting in skin inflammation (Bornstein *et al.* 2001, Pence and Ueckermann 2002, Arlian and Morgan 2017), causing a suite of effects that fall into two broad categories: physiological and behavioural. Physiological effects encompass changes in metabolism, skin condition (Pence and Ueckermann 2002), reproduction (Fthenakis *et al.* 2001, Sarasa *et al.*

2011, Laha 2015), biochemistry (Dimri *et al.* 2008, De and Dey 2010, Saleh *et al.* 2011), growth (Newman *et al.* 2002, Serrano *et al.* 2007), thermoregulation (Cross *et al.* 2016, Simpson *et al.* 2016), immunology (Sarasa *et al.* 2010), and body condition (Newman *et al.* 2002, Ruykys *et al.* 2009). Behavioural effects include irritation (Pence and Ueckermann 2002), as well as changes in foraging (Simpson *et al.* 2016), home range and dispersal (Chronert *et al.* 2007, Soulsbury *et al.* 2007), and circadian rhythmicity (Overskaug 1994, Borchard *et al.* 2012). These effects have complex interactions, whereby one disease condition can cause, influence, or amplify another, resulting in cascading and compounding pathogenic impacts on the host (Figure 2.1).

Although a range of characteristics connecting *S. scabiei* infection to mange disease is known, important knowledge gaps remain. Evidence suggests that the energetic burden of mange must be substantial, as weight loss is commonly observed in mange infected hosts (Bornstein *et al.* 2001, Pence and Ueckermann 2002); however, the metabolic impacts of *S. scabiei* infection are poorly understood. It is possible that *S. scabiei* infection may increase host metabolism owing to the costs of mounting an immune response. Evidence also suggests that there may be metabolic costs of alopecia and associated thermoregulation (Cross *et al.* 2016, Simpson *et al.* 2016), but investigations regarding this topic remain limited. The host may attempt to compensate for the aforementioned changes through shifts in behaviour (e.g., to save energy or increase energy intake). However, evidence is inconsistent about the nature of these shifts (Verstegen *et al.* 1987, Simpson *et al.* 2016), and information is needed that encompasses full circadian rhythms. *Sarcoptes scabiei* infection can result in a loss of animal condition through depletion of fat stores (Newman *et al.* 2002, Lau *et al.* 2007, Ruykys *et al.* 2009), but infection may also cause an imbalance of fatty acid composition across tissues (e.g., brain, bone marrow, and muscle tissues), that contributes to function (e.g., immune function, motor skills, behaviour), which is less well recognised.

Wombats are an important example of a host species for which *S. scabiei* has become an emerging disease, and continues to cause significant pathology (Skerratt 2005, Martin *et al.* 2018a). Consistent with other host species experiencing crusted mange disease, infected wombats experience skin fissures and hyperkeratosis (Skerratt 2003b, Hartley and English 2005, Ruykys *et al.* 2013), reduced fat stores and emaciation (Martin *et al.* 1998, Skerratt 1998, Ruykys *et al.* 2009), loss of body condition (Skerratt *et al.* 1999, Skerratt *et al.* 2004b, Ruykys *et al.* 2009), decreased reproductive function (Skerratt *et al.* 1999, Ruykys *et al.* 2009), and higher

thermal differentials (Simpson *et al.* 2016). Behaviourally, they exhibit increased diurnal activity (Hartley and English 2005, Ruykys *et al.* 2009, Borchard *et al.* 2012), may travel farther (Skerratt *et al.* 2004b), spend more time outside of the burrow (Simpson *et al.* 2016), and reallocate the amount of time devoted to different behaviours (Simpson *et al.* 2016).

Here, we develop the body of knowledge around the cascading consequences of mange disease manifestation, focussing on the Tasmanian bare-nosed wombats (*Vombatus ursinus*). We address critical knowledge gaps linking the cascading effects of infection that manifest in disease, broadly encompassed within the disciplines of integrative biology and conservation physiology. Specifically, we aim to: (i) quantify heat loss in mange infected wombats, (ii) calculate field metabolic rates for diseased and healthy individuals, (iii) assess behavioural changes across full circadian cycles, specifically foraging and resting behaviours, and whether these are sufficient in compensating energy demands of disease, and, (iv) quantify fatty acid composition across tissues in mangy wombats. Due to the challenges associated with obtaining these types of physiological and behavioural data in free-living animals, we have exploited multiple data sources. Our sample sizes are necessarily moderate, but provide valuable insight to the powerful impacts of *S. scabiei* on its hosts.

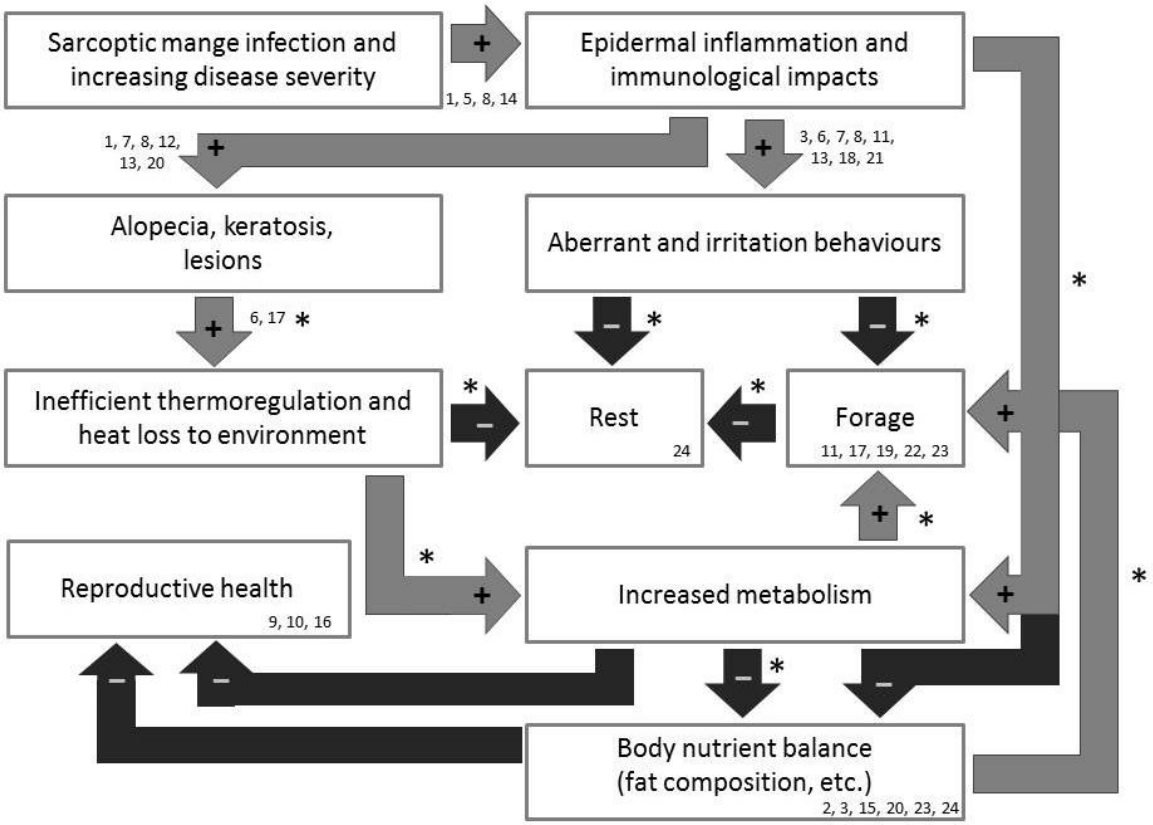


Figure 2.1. Cascading impacts of *S. scabiei* infection causing mange disease manifestation. (*) Denotes disease associations investigated in this study. (1) Arlian & Morgan 2017, (2) Beigh *et al.* 2016, (3) Beigh *et al.* 2013, (4) Borchard *et al.* 2012, (5) Bornstein *et al.* 2001, (6) Cross *et al* 2016, (7) Cypher *et al.* 2017, (8) Diwakar & Diwakar 2017, (9) Fthenakis *et al.* 2001, (10) Laha 2015, (11) Murray and Cassidy St. Clair 2017, (12) Nimmervoll *et al.* 2013, (13) Oraon *et al.* 2000, (14) Pence & Ueckerman 2002, (15) Pérez *et al.* 2015, (16) Sarasa *et al.* 2011, (17) Simpson *et al.* 2016, (18) Skerratt *et al.* 2004a, (19) Skerratt *et al.* 2004b, (20) Skerratt 2003b, (21) Süld *et al.* 2017, (22) Süld *et al.* 2014, (23) Tataruch et al 1985, (24) Verstegen *et al.* 1987.

2.3 Methods

2.3.1 Scoring of mange severity

Mange severity scoring followed the protocol described by Simpson *et al.* (2016) whereby each individual is divided into 14 body segments, seven segments on each side: head (H), shoulder (Sh), forelimb (FL), stomach (St), back (B), hind limb (HL), and rear(R). Each segment is assigned a score reflecting hair loss, from 0 (no hair loss) to 10 (>70%) (Supplementary Material I). The average mange severity score is the mean of the segment scores (with the exception of section 2.2, where mange severity is the average of the forelimb, stomach, and hind limb, only). Due to the asymmetric nature of alopecia in mange infected wombats, averaged mange severity scores are typically much lower than the most severely infected individual segment score. Mange severity scores ranged from completely healthy (lowest severity score: 0) to late stage mange (highest severity score: 7.5)(Martin *et al.* 2018a).

2.3.2 Aim I -Quantifying heat loss

To understand the energetic cost of mange induced hair loss on wombat thermoregulation, we used thermal imagery to calculate heat loss (W/m^2). Thermal imaging has been a powerful and non-invasive tool in studying thermoregulation and thermal physiology, performing population surveys and count surveys, and diagnosing disease (McCafferty 2007). This method has been used to both diagnose (Arenas *et al.* 2002) and quantify the impact (Cross *et al.* 2016) of sarcoptic mange in wildlife.

Five free-living wombats at Narawntapu National Park (NNP; Tasmania, Australia, -41.15°N, 146.60°W) were opportunistically photographed using the Testo 870-1 Thermal Imager (thermal sensitivity < 0.1 K, 32˚ field of view, 320 x 240 pixels) from March through June of 2014 (Supplementary Material II). Individuals ranged from healthy (highest individual segment scores of 0−2, n=2) to late stage mange infected (highest segment scores of 9−10). Photographs were taken manually, with replicate photographs taken of each individual to capture various angles (with profile angles being optimal for viewing all body segments; Figure 2.2). Hourly ambient temperature was collected by the nearest weather station in Devonport, TAS (Devonport Airport, -41.17°N, 146.42°W; 10 km east of NNP), and ranged between 9.7˚C

– 18.9˚C. Images were processed using software (Testo IRSoft v.3.1) to calculate maximum surface temperatures for each body segment. The temporal span of photos of healthy and mange infected wombats overlapped, such that any effects of mange are not confounded by time of day. Indeed, most images were taken during the evening. Due to the diurnal behaviour of mange infected wombats (Simpson *et al.* 2016), some images were necessarily taken during daylight hours; many others in overcast weather. Also, to avoid possible impacts by solar radiation, the shoulder, back, and rear segments were not used for analyses. Regardless, hair loss in mange infected wombats is predominately observed in the forelimb, stomach, and hind limb segments, and thus the omission of the shoulder, back, and rear from analyses was inconsequential for examining the thermal energetic costs of *S. scabiei* infection. Average mange severity scores for this analysis were the average of the mange scores from the forelimb, stomach, and hind limb, only (Figure 2.2). Heat loss was defined as the sum of convective (free and forced) and radiative heat loss, following methods by Cross *et al.* (2016) (Supplementary Material III). Linear regressions were used to understand the relationship between mange score (balding %) and heat loss (W/m^2) for the FL, St, and HL segments. Heat loss (W) can be transformed into kJ per hour per segment using the segment area and the conversion of 1 W to 3.6 kJ per hour. Total heat loss (per hour) for each wombat is the sum of heat loss in the forelimb, stomach, and hind limb.

2.3.3 Aim II – Field metabolic rates

The Doubly Labelled Water (DLW) technique was used to estimate field metabolic rates and water turnover rates in wombats (Lifson *et al.* 1955, Lifson and McClintock 1966, Evans *et al.* 2003). Our methodology followed the two-sample DLW protocol whereby: 1) a background blood sample is taken; 2) the body water pool is enriched with hydrogen and oxygen isotopes (equilibration); 3) a second blood sample is taken after isotope equilibration with body water pool; and 4) a final blood sample is taken after 1–2 biological half-lives of the oxygen isotope. Carbon dioxide production can be estimated from the amount of isotope depleted over time (Nagy 1983).

A total of nine individuals (8 adults, 1 juvenile) were trapped at NNP between April – June 2015 to assess field metabolic rates (Table 2.1). Individuals were trapped on foot using large, mesh

nets and were anaesthetized (zolazepam/tiletamine, Zoletil, Virbac, dose: 3-4mg kg^{-1} and medetomidine, dose: 40µg kg^{-1} intramuscular [IM] injection; Ethics Approval Permits A14670, FA15122). Individuals were weighed and the initial blood draw was taken, followed by an intra-peritoneal injection (IP) with 4 mL of ^{18}O (≥98%) and 4 mL of deuterium (>99.9%) following Evans *et al.* (2003). Wombats remained under light anaesthesia (with re-administration of zolazepam/tiletamine and medetomidine on a per wombat basis) during the equilibration period after the initial blood draw and isotope injection, and through the second blood draw (taken ~4 hours after isotope injection, and ~4.5 hours post initial blood draw) (Evans *et al.* 2003). Post-processing, wombats were administered a sedative reversal (atipamezole, dose: 40µg kg^{-1} IM) and held in wire Mascot animal traps, padded and insulated with hessian sacks, for 6−12 hours until fully recovered from anaesthesia. Wombats were released at the site of capture. Recapture efforts for final blood draw were focused 1−2 ^{18}O biological half-lives after initial capture (10−14 days; Evans *et al.* 2003). Eight of the nine individuals were recaptured for the final blood draw, all 8 to 13 days after initial capture.

Blood samples from the initial (background and post-equilibration) and final captures were sent for deuterium and ^{18}O enrichment analyses (Metabolic Solutions, Inc, New Hampshire, USA). Enrichments were used to calculate water flux and CO_2 production (mL CO_2 g^{-1} h^{-1}) using equations from Nagy (1983). Field metabolic rates (kJ day^{-1}) were calculated from CO_2 production using the factor 21.8 J ml^{-1} CO_2, which was derived from a koala leaf diet (Nagy and Martin 1985, Evans *et al.* 2003). Lastly, feeding rates (required amount of dry plant matter intake to meet metabolic needs) were calculated using the metabolisable energy available through the wombat diet, which is estimated to be 7.4 kJ g^{-1} (Evans *et al.* 2003). A linear regression was used to understand the relationship between mange severity score and metabolic rate. The one juvenile wombat captured was not included in the linear regression analysis, due to different energetic requirements for juveniles relative to adults.

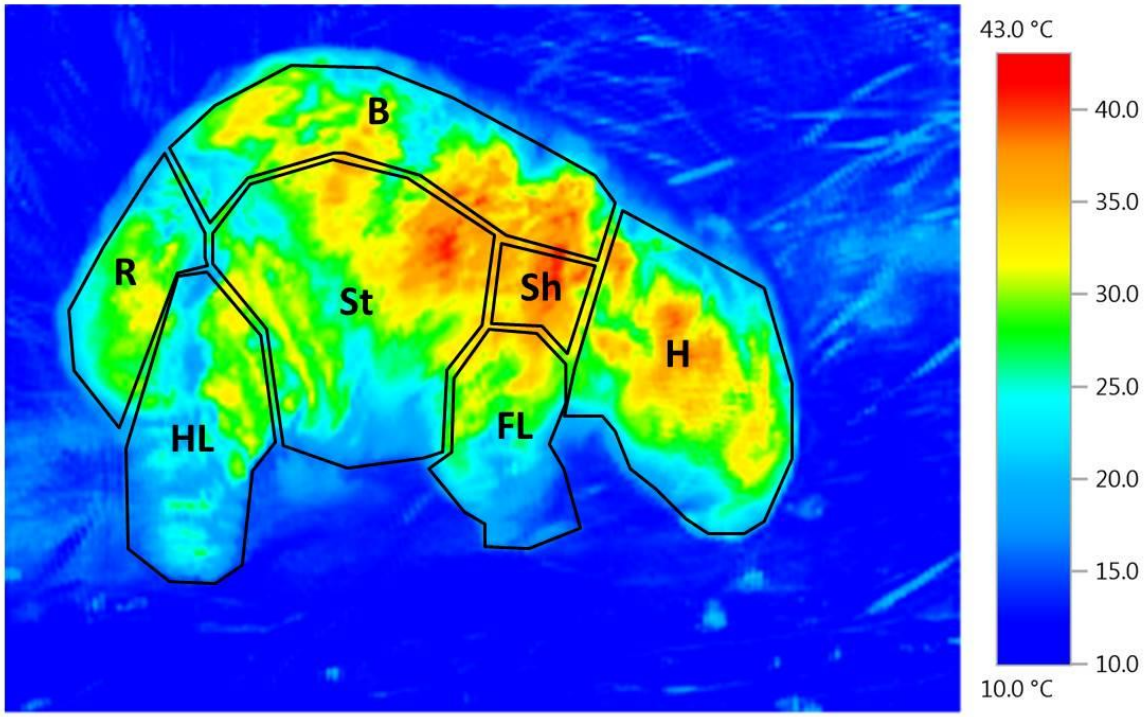


Figure 2.2. Thermal image of a wombat with the seven segments defined. Each segment was assigned a mange score based on methods described in Simpson *et al*. (2016), and the maximum temperature from each segment was documented. Forelimb (FL), stomach (St), and hind limb (HL) were used for heat loss analyses, while rear (R), back (B), shoulder (Sh), and head (H) were excluded.

2.3.4 Aim III – Resting and foraging behaviour

To assess disease induced behavioural differences in wombats, triaxial accelerometer data loggers (AX3 Axivity) were deployed on five adult, free-living wombats in NNP (one healthy, one with ambiguous signs of early mange, and three mange-infected). Wombat trapping and processing followed the protocols outlined above (see 2.3.3). All loggers were set out within 24-hours of each other, on 20– 21st of April 2015. Three loggers were successfully retrieved: one from a moderately mange infected wombat (female, W006, mange severity score 2.7), one from a wombat with ambiguous signs of early mange (male, W009, mange severity score 0.57, referred to as "early"), and one from a healthy wombat (female, W002, mange severity score 0.5). Despite having a similar average mange score to W002, W009 had ambiguous signs of early mange at capture (with confirmed mange in subsequent visual surveys), and thus, was conservatively classified as early stage mange. The loggers recorded at 50 Hz from noon on 22 April 2015 to varying times on 18 May 2015.

Traces for the 3 cardinal axes of the accelerometer were visualised in Somnologica Studio 3.0 and activities were defined based on stereotypic patterns. To calibrate real-time wombat activities with accelerometer recordings, Axivity data loggers were also deployed on two healthy, captive wombats. Based on captive wombat accelerometer recordings, six main activities were identified: digging, steady walking, scratching, running, slow walking/ grazing, and inactivity. In addition, there were four unidentified activities and an activity categorized as "restlessness," which was defined as a period of brief, unrecognizable activity interrupting periods of inactivity. Activities were manually scored in 3-second epochs (28,800 epochs per day), and each epoch was categorized as the activity that endured for the majority of that epoch. Activities were scored for four, 24-hour periods (at three day intervals, excluding the first 72 hours post-anaesthesia: 24 April, 27 April, 30 April, 03 May) for each wombat (115,200 epochs individually scored per wombat, or 345,600 total).

Inactivity and foraging behavioural data were quantified in three ways: total number of episodes per behaviour (per day), average duration of activity bouts, and percentage of day spent engaged in either state (for daily activities and averages, see Supplementary Material IV and V). For the average duration of activity bouts, bouts were defined as either an isolated epoch of activity (3 seconds), or consecutive epochs of the same activity (>3 seconds).

Differences in the number of episodes, bout durations, and the proportion of time spent engaged in inactivity and foraging were analysed among wombats using ANOVAs. Inter-individual differences were assessed using a multi-comparison of means (Tukey Contrasts). Plots of daily wombat activity from 12:00pm on 22 April to 05:00 on 08 May show differences in circadian cycles (Supplementary Material VI).

To assess whether mange infected wombats can cope with the metabolic pressures of mange, realized feeding rates (energy consumed [kJ day^{-1}], derived from behavioural data) were also calculated. The average proportion of the day spent foraging (for the healthy and late stage wombats) was used, in combination with bite rates derived from Simpson *et al*. (2016) for healthy wombats and mange infected wombats, to determine the number of bites taken per day. The amount of dry plant matter per wombat bite was assumed to be 0.015 g, along with 7.4 kJ metabolisable energy per gram of dry plant matter (Evans *et al.* 2003).

2.3.5 Aim IV – Fat composition

Fatty acid composition was analysed in four tissue types (adipose, brain, bone marrow, muscle) from eight Tasmanian wombats, which were euthanized owing to injuries from vehicle collision or severe mange disease, between 2015 and 2016 (Supplementary Material II). Wombats were given a mange score and condition assessment prior to sampling. Average mange severity scores ranged from 0 (completely healthy) to 7.5 (late stage mange infection). Muscle was obtained from the shoulder region, bone marrow from the femur, and adipose subcutaneously.

Fat composition profiles were calculated by the National Measurement Institute (NMI) for each tissue type using Fatty Acid Methyl Esters (FAME). Proportions of FAMEs are relative to the amount of sample tissue (0.5−10 grams), and were determined by gas chromatography. Principle Component Analyses (PCA) were performed for each tissue type to select for fatty acids that had the best explanatory power (loading values ≥0.3). Linear regressions were then run for each of the four tissues, using the results from PC1 and the mange severity scores.

2.4 Results

2.4.1 Aim I – Sarcoptic mange and heat loss

Mange severity scores varied within individual wombats across body segments (Figure 2.3). All body segments showed a positive relationship between mange severity and heat loss, with stronger relationships shown in the stomach (R^2=0.75, $F_{1,3}$=12.74, *P* =0.04), and more moderate relationships for the forelimb (R^2=0.50, $F_{1,3}$=5.01, *P* =0.11) and the hind limb (R^2=0.49, $F_{1,3}$=4.91, *P* =0.11; Figure 2.3).

2.4.2 Aim II – Mange and host water flux and field metabolic rate

There was a significant positive relationship between average mange score and metabolic rate (kJ kg^{-1} day^{-1}) (R^2=0.59, $F_{1,5}$=9.5, *P* =0.03; Figure 2.4). This trend continues to hold true when the regression is run with adult females only (R^2=0.89, $F_{1,3}$=36.38, *P* <0.01). No significant relationship was observed between average mange score and water influx (mL kg^{-1} day^{-1}) (R^2=-0.17, $F_{1,5}$=0.13, *P* =0.74). On average, individuals infected with mange experienced a 40% increase in their field metabolic rate compared to healthy wombats (155.4 kJ kg^{-1} day^{-1}, 218.3 kJ kg^{-1} day^{-1}, respectively).

Based on the results and assumptions of food calorific content, healthy wombats (with a highest segment score of ≤2; n=5) would need to consume 452 grams of plant matter (± 30.54 g) per day to meet their metabolic requirements, while wombats with early signs of mange (highest segment score of ≥3, n=2) would require 602 grams per day (± 80.43 g). This equates to 33.2% greater food requirement for animals with signs of early stage mange compared to healthy individuals.

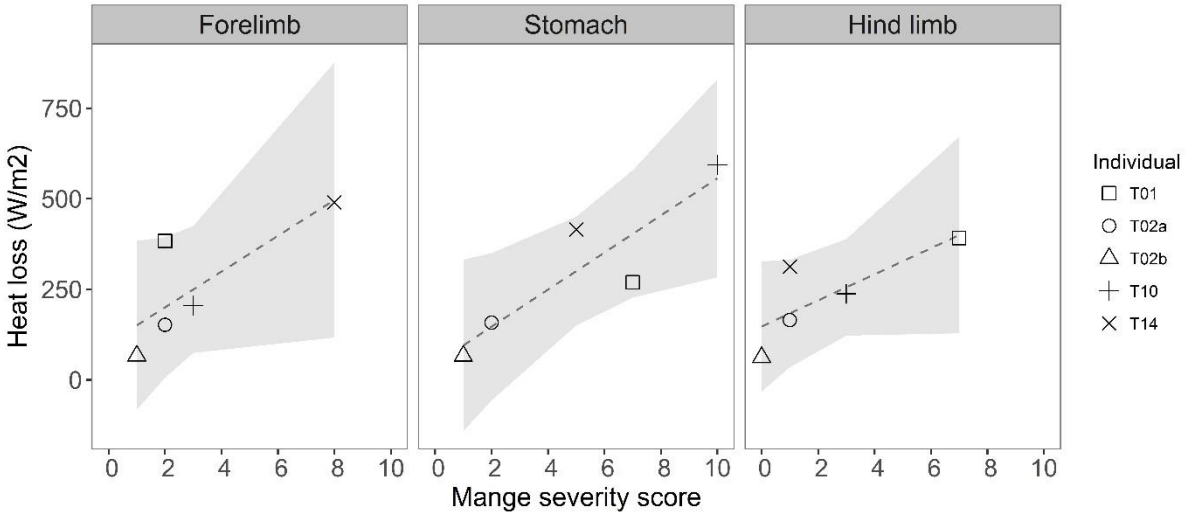


Figure 2.3. Heat loss (W/m^2) from three body segments in healthy and mange-infected wombats (n=5) with 95% confidence intervals (grey). As the segment mange score increased, the amount of heat lost increased in the forelimb (R^2=0.50, $F_{1,3}$=5.01, *P* =0.11), stomach (R^2=0.75, $F_{1,3}$=12.74, *P* =0.04), and hind limb (R^2=0.49, $F_{1,3}$=4.91, *P* =0.11). Individuals are identified to show the variation in mange severity across body locations, reflecting infection asymmetry and inter-individual variation.

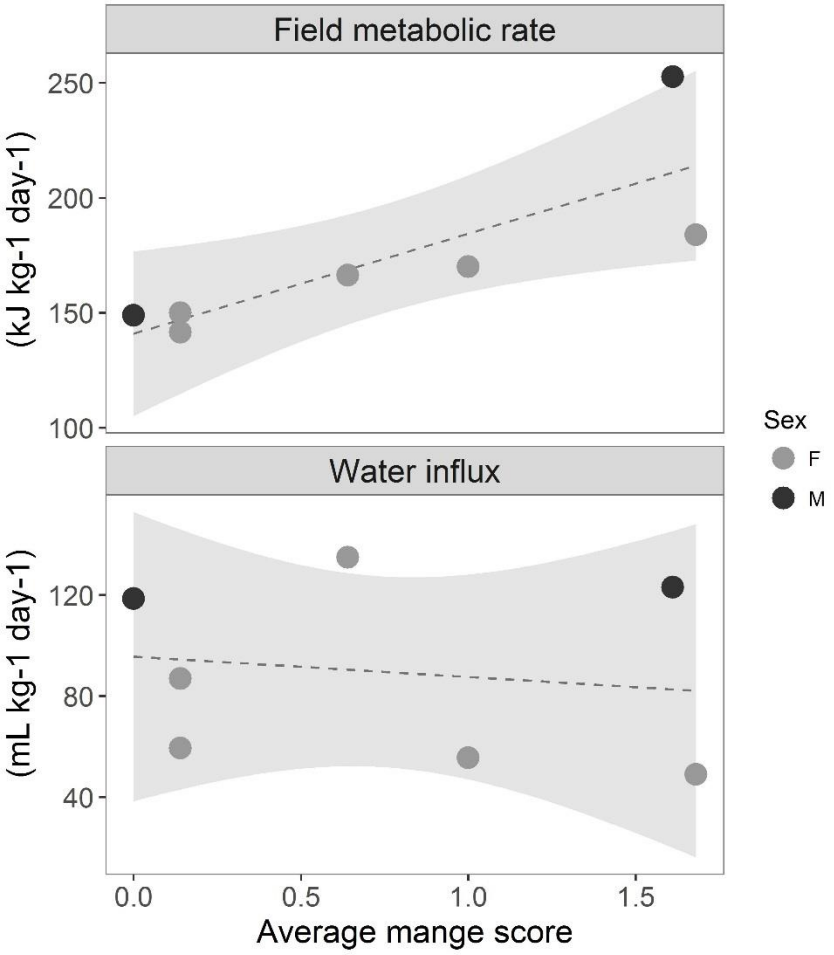


Figure 2.4. The effect of mange severity on wombat field metabolic rate (kJ kg^{-1} day^{-1}). Seven adult wombats (five female, two male) at varying mange severities were used to assess field metabolic rate. The average mange severity score is the mean of the body segment scores, as described by Simpson et al. (2016). As average mange severity increased, metabolic rate increased (R^2=0.59, $F_{1,5}$=9.57, *P*=0.03). This trend holds true when females are analysed separately (R^2=0.89, $F_{1,3}$=36.38, *P*<0.01).

2.4.3 Aim III – Mange induced changes in foraging and inactivity

The mange infected wombat spent significantly less time grazing during the 24-h day than healthier animals ($F_{2,6}$=13.33, P<0.01; healthy, 39.8% ± 1.0 S.E.; early, 35.2% ± 1.0 S.E.; moderate, 27.0% ± 2.7 S.E.) (Figure 2.5). This reduction in grazing arose from shorter grazing bouts that were one-third the duration as that observed in the healthiest animal ($F_{2,12133}$=208.6, P<0.01; healthy, 62.6 sec. ± 1.3 S.E.; early 23.7 sec. ± 0.2 S.E.; moderate, 19.4 sec. ± 0.1 S.E.). Those with the highest mange scores engaged in more grazing episodes than the wombat with the lowest mange score ($F_{2,6}$=18.89, P<0.01; healthy, 549.5 ± 53.7 S.E.; early, 1284.3 ± 103.7 S.E., moderate, 1201.0 ± 186.1 S.E.). Conversely, the amount of inactivity increased with the average mange score ($F_{2,6}$=8.85, P=0.02; healthy, 53.1% ± 1.2 S.E.; early, 57.8% ± 1.8 S.E.; moderate, 63.5% ± 2.6 S.E.). This pattern was mirrored by an increase in the number of episodes of inactivity, indicating that more diseased animals had more episodes of inactivity ($F_{2,6}$=31.19, P<0.01; healthy, 594.5 ± 87.0 S.E.; early, 1081.3 ± 30.4; moderate, 1613.8 ± 142.4), yet these episodes were of a shorter duration ($F_{2,13152}$=67.10, P<0.01; healthy, 77.2 sec. ± 1.5 S.E.; early, 46.2 sec. ± 1.0 S.E.; moderate, 34.0 sec. ± 0.3 S.E.). The individual with the highest mange score spent the most amount of time scratching (Supplementary Material IV and V).

Realized feeding rates differed between healthy and mange infected wombats. The healthy wombat spent about 39% of the day foraging, while, the mangy wombat only spent about 27% of the day foraging (using data from W006, only) (Supplementary Material V). Additionally, healthy wombats have bite rates of 84 bites per minute, and mange infected wombats have an estimated bite rate of 71 bites per minute (Simpson *et al.* 2016). With these foraging and bite rates, healthy wombats are able to consume 748.2 grams of plant matter per day (equivalent to 5536 kJ day^{-1}), which is sufficient to meet or exceed their daily energy requirements (3344.9 kJ day^{-1} ± 226 S.E.; Table 2.1). Conversely, mange infected wombats can ingest approximately 439.5 grams of plant matter per day (equivalent to 3252 kJ day^{-1}), falling short of their daily needs (4457.4 kJ day^{-1} ± 595.2 S.E.; Table 2.1).

Table 2.1. Water turnover, metabolic, and feeding rates of *Vombatus ursinus* (n=8). Averages are calculated for adults grouped as healthy (highest segment score ≤2) and early disease stage (highest segment score ≥3).

Animal					BM	Total Body Water		Water Turnover		Field Metabolic Rate			Feeding Rate
ID	Sex	RS*	Age	Average mange severity score	g	g	%	Efflux mL kg^{-1} day^{-1}	Influx mL kg^{-1} day^{-1}	mL CO2 g^{-1} h^{-1}	kJ day^{-1}	kJ kg^{-1} day^{-1}	g plant matter day^{-1}
10	F	n/a	J	0.00	8000	5536.2	0.69	92.04	98.39	0.363	1609.12	189.31	217.45
11	F	R	A	0.14	19500	13100.1	0.67	86.90	86.90	0.288	2925.43	150.02	395.33
12	M	n/a	A	0.00	26000	19253.7	0.74	119.92	118.59	0.286	3833.08	148.86	517.98
17	F	n/a	A	0.64	21000	16556.3	0.79	130.87	134.99	0.319	3575.38	166.30	483.16
18	F	R	A	0.14	19000	13269.9	0.70	59.40	59.40	0.272	2690.53	141.61	363.59
22	F	n/a	A	1.00	22000	15736.5	0.72	57.71	55.61	0.327	3700.07	170.12	500.01
Mean								90.96	91.10	0.298	3344.90	155.38	452.01
S.E.								15.08	15.75	0.01	226.02	5.47	30.54
16	M	n/a	A	1.61	20000	15195.6	0.76	123.12	123.12	0.485	5052.64	252.63	682.79
19	F	R	A	1.68	21000	15787.9	0.75	49.07	49.07	0.353	3862.21	183.91	521.92
Mean								86.09	86.09	0.419	4457.42	218.27	602.35
S.E.								37.03	37.03	0.07	595.21	34.36	80.43

**Reproductive status (R represents a reproductive adult)*

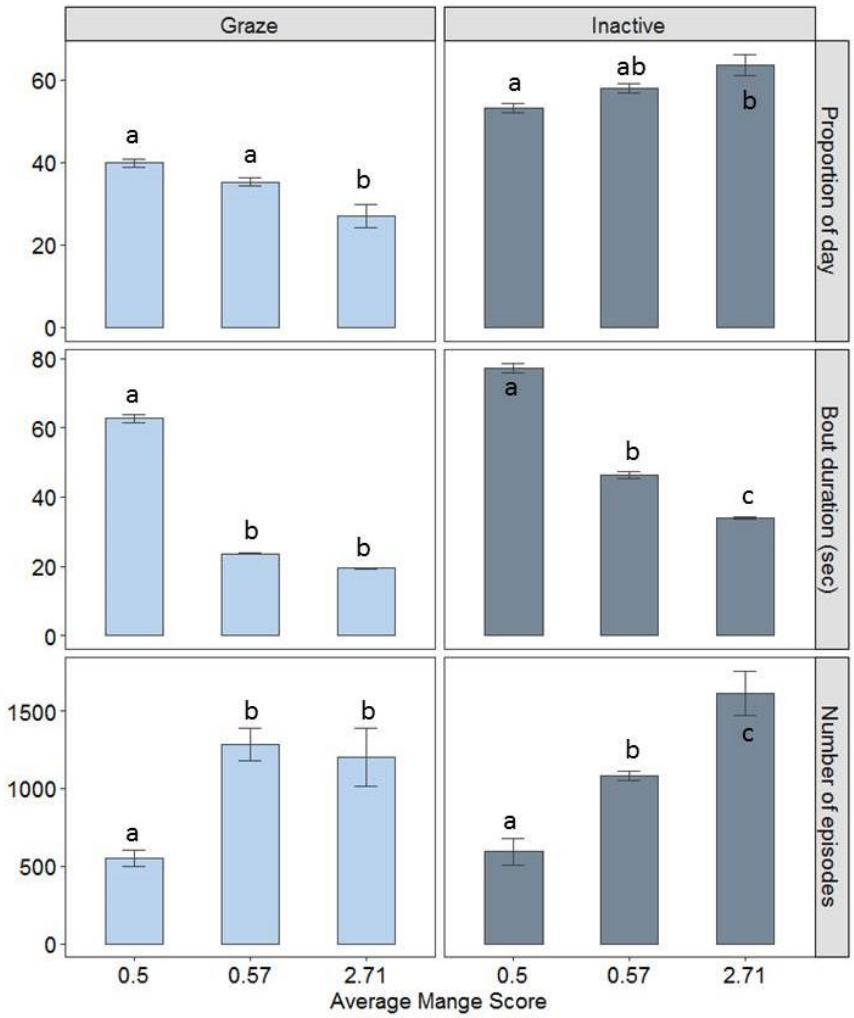


Figure 2.5. Mange induced changes in bare-nosed wombat grazing and inactivity behaviours. Three wombats, one healthy (mange score 0.5), one with ambiguous signs of early mange (mange score 0.57), and one with moderate mange (mange score 2.71), were observed for grazing and inactivity behaviours across four full circadian cycles (days). Each day was composed of 28,800 3-second epochs, and each epoch was assigned to the activity that lasted the majority of the three seconds, giving a total of 345,600 epochs manually scored. Behavioural changes were analysed using three types of data: average number of episodes per activity per 24-h day, average bout duration per activity, and proportion (%) of day spent engaging in each activity. Significant differences between values are indicated by labels “a”, “b”, and “c.”

2.4.4 Aim IV – Fatty acid composition

A total of 34 fatty acids were incorporated into a PCA, as well as fat sums (e.g., total monounsaturated, total polyunsaturated) (Supplementary Material VII). The PCA results revealed 14 fatty acids to have the strongest explanatory power: palmitic, stearic, oleic, eicosenic, omega 6 (n-6), omega 3 (n-3), n-6 linoleic, n-3 alpha-linolenic, n-6 arachidonic, n-6 docosatetraenoic, n-3 docosahexaenoic, total polyunsaturated, total saturated, and total monounsaturated. The relationship between average mange scores and the proportion of these 14 fatty acids present in each tissue type are represented visually (Supplementary Material VIII).

These 14 fatty acids were then used in a more restricted PCA (Table 2.2), for which PC1 was evaluated as a linear response variable to wombat mange severity score for each tissue type (Figure 2.6). There was a significant relationship between adipose fatty acid composition and mange severity ($F_{1,6}$=6.40, P=0.04, R^2=0.44), but no relationship between fatty acid composition and mange severity in the bone marrow, brain, and muscle tissues ($F_{1,6}$=1.25, P=0.30, R^2=0.03; $F_{1,6}$=1.04, P=0.35, R^2=0.01; $F_{1,6}$=0.73, P=0.43, R^2=-0.04, respectively). The adipose fatty acid results suggest that as mange severity increases, omega-6 and arachidonic acid (C20:4) increase, and oleic acid (C18:1), alpha linoleic acid (C18:3), and total monounsaturated fats decrease (Table 2.2, Figure 2.6).

Table 2.2. Principle component analysis results from fat composition in four wombat tissues. PC1 loading values are presented for 11 individual fatty acids and three acid type summations (total polyunsaturated, total saturated, total monounsaturated). PC1 loading values ≥0.3 in bold.

Fatty Acid	Type *	Adipose 83.8%	Bone marrow 55. 4%	Brain 86.8%	Muscle 75.1%
C16:0 Palmitic	SFA	-0.19	**0.31**	-0.17	0.22
C18:0 Stearic	SFA	0.24	**-0.31**	0.02	0.02
C18:1 Oleic	MUFA	**-0.31**	**0.35**	0.29	0.16
C20:1 Eicosenic	MUFA	0.01	-0.01	**0.33**	0.00
Omega-6	PUFA	**0.55**	**-0.43**	-0.05	**-0.56**
C18:2 n-6 Linoleic	PUFA	0.12	-0.10	-0.11	**-0.52**
C20:4 n-6 Arachidonic	PUFA	**0.30**	-0.14	0.02	-0.04
C22:4 n-6 Docosatetraenoic	PUFA	0.04	-0.02	0.04	0.00
Omega-3	PUFA	-0.28	-0.09	**-0.35**	0.09
C18:3 n-3 alpha Linolenic	PUFA	**-0.39**	0.06	-0.24	0.11
C22:6 n-3 Docosahexaenoic	PUFA	0.02	-0.02	-0.07	-0.01
Total polyunsaturated	-	0.27	**-0.51**	**-0.40**	**-0.47**
Total saturated	-	0.04	0.07	-0.21	0.26
Total monounsaturated	-	**-0.32**	**0.44**	**0.60**	0.19

**Saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA)*

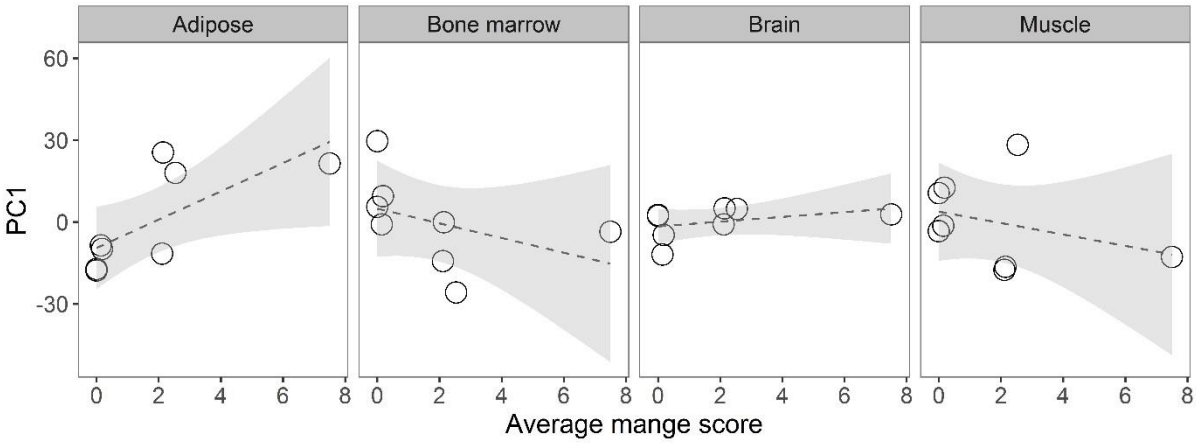


Figure 2.6. The relationship between average mange severity score and the fatty acid composition of four tissue types. Regressions were run for each tissue type using PC1 predictor values (from PCA of 14 fatty acids, Table 2.2) and average mange severity scores. There was a significant relationship between adipose fatty acid composition and mange severity ($F_{1,6}$=6.40, P=0.04, R^2=0.44), but no significant relationship in other tissues (bone marrow $F_{1,6}$=1.25, P=0.30, R^2=0.03; brain $F_{1,6}$=1.04, P=0.35, R^2=0.01; muscle $F_{1,6}$=0.73, P=0.43, R^2=-0.04).

2.5 Discussion

The term 'disease' is a manifestation of all the consequences of infection. The impact of infection can be diverse, direct or indirect, difficult to measure, and can have cascading interactions resulting in amplified effects. Sarcoptic mange is an emerging infectious disease of mammals (Tompkins *et al.* 2015), affecting more than 100 species worldwide (Arlian and Morgan 2017), with generally conserved effects across species. Thus, addressing the individual and compounding effects of mange-induced changes in one host may have implications for other host species. Here, we bridge critical knowledge gaps regarding the impacts of physiological changes on host metabolism, thermal energetic demands, effects on host behaviour, and fat composition. Specifically, we show that mange infected wombats experience heightened energetic demands through heat loss and raised metabolism. We find that wombats cannot compensate for the increased metabolic requirement through altering foraging behaviours (indeed they spend more time inactive), and subsequently deplete their fat stores, with altered fatty acid composition in adipose tissues, but not necessarily other tissues. These findings improve our understanding of the process by which *S. scabiei* infection results in host physiological changes, progressive disease phenotypes, and mortality; and, may also contribute to other globally important chronic inflammatory parasitic infections of animals, such as notoedric mange (Foley *et al.* 2016).

In mammals, hair plays a major role in the conservation of energy and regulation of the daily energy budget. However, disruption of the pelt-environment interface by means of alopecia (e.g., from *S. scabiei* infection) can result in inefficient thermoregulation and excessive heat loss to the environment through the skin. This can be particularly impactful when alopecia is substantial (>50%) across the host body. Alopecia with subsequent heat loss is observed in a range of parasite systems (e.g., ticks on moose (Addison and McLaughlin 2014); *S. scabiei* in wolves (Cross *et al.* 2016); *Demodex* spp. mites in mule deer (Gentes *et al.* 2007)), with implications for increased energetic burden on the host. We found that infected wombats can lose between ~140 – 235 kJ per hour (1.1 – 1.8MJ per day, assuming eight hours of activity) through their alopecia-impacted forelimb, stomach, and hind limb, while healthy wombats lose as little as ~40 – 90 kJ per hour (0.3 – 0.7 MJ per day). This translates to 1.56 – 5.88 times more energy loss. Additionally, this energy burden may be much higher (per day) in mange infected wombats, due to their tendency to spend more time outside of the burrow (Simpson *et al.*

2016), and thus experience increased conductive heat loss to flowing air. However, this potential increased cost may be ameliorated through their shift toward diurnal activity (Borchard *et al.* 2012). Despite the likely underestimation of heat loss in mange infected wombats due to the use of only three body segments, these rates are comparable to those of small wolves (around twice the mass of wombats) with early mange, which lose approximately 3.5 – 6.5 MJ per night (Cross *et al.* 2016). These findings provide insight into the energetic cost of alopecia and heat loss in mange infected wombats, and suggest that heat loss may play a major role in changes to metabolism (see below).

We found that the compounding impacts of host responses to *S. scabiei* infection have metabolic consequences that the host cannot sustain long-term. There is broad consensus that prompting an immune response is energetically expensive (Lochmiller and Deerenberg 2000, Bonneaud *et al.* 2003), and this energetic burden can also be exacerbated through physiological changes. Wombats infected with mange experienced a 40% increase in their field metabolic rate compared to healthy wombats (155.4 kJ kg^{-1} day^{-1}, 218.3 kJ kg^{-1} day^{-1}, respectively), a rate that would require infected individuals to consume on average ~150 grams more of plant matter per day to meet their metabolic needs. While these field metabolic rates fall within the documented range for mainland bare-nosed wombats during the wet season (Evans *et al.* 2003), the baseline metabolic requirements for individuals living in Tasmania are likely to be lower than those from mainland Australia, due to cooler seasonal daily temperatures (Brown *et al.* 2004). Survival of mange infected wombats with increased energetic demands will depend on their ability to increase their energy intake, and may require behavioural plasticity.

The energetic and physiological effects of disease presence can also induce host behavioural changes (Bonneaud *et al.* 2003, Bradley and Altizer 2005, Martín-Hernández *et al.* 2011, Simpson *et al.* 2016), through direct or indirect impacts. For example, a host impacted by disease may adjust behaviours due to direct impacts, such as reduced mobility or function, or due to indirect impacts, such as engaging in new activities in response to the disease that necessarily decrease the time available for other behaviours. Our findings add to previous research and suggest that *S. scabiei* infected wombats attempt to increase their foraging effort to compensate for the energetic demands of mange; but, here we show why they are unsuccessful in doing so.

Mange infected wombats engage in more periods of foraging behaviour than healthy wombats, but are unable to sustain foraging efforts for extended periods of time, resulting in a smaller proportion of the day spent foraging, overall. Additionally, mangy wombats are unable to engage in extended periods of rest, likely owing to the epidermal irritation caused by the mite (Bornstein *et al.* 2001, Pence and Ueckermann 2002). Similar to foraging, mange infected wombats engage in more periods of inactivity, but unlike the decrease in foraging observed in mangy wombats, inactivity increased in those with the highest mange score. The inability for diseased wombats to maintain periods of both foraging and inactivity may be due to interruption by mange related activities, such as scratching. Indeed, wombats with more severe mite infestations spend more time scratching than healthier animals (Skerratt 2003b, Simpson *et al.* 2016). Combined, our results suggest that when the energetic pressure of mange is too high, the host may not be able to compensate. In this case, the daily energy intake required to survive exceeds the metabolisable daily energy intake rate. Furthermore, shorter and interrupted periods of inactivity may reduce their ability to conserve energy by resting (as seen in other species; Verstegen *et al.* 1987). It is important to note that these conclusions have been derived from a modest sample size, and further investigation into mange-induced host behavioural changes would greatly improve our understanding of the disease.

We were also motivated to understand if *S. scabiei* infection alters fat composition across tissues, and thus, impacts functions that could be connected to other aspects of mange disease (e.g., behaviour). When hosts cannot ameliorate energetic demands of disease, they must draw on energy stores to survive. The most obvious consequence of this is emaciation, with less obvious impacts on fat composition in vital tissues, which feed back into the cascade of disease impacts. We found that effects of *S. scabiei* infection on fat composition were most obvious in adipose tissues, where increased levels of n-6 arachidonic acid and decreased levels of countering n-3 fatty acids (alpha linolenic) were observed in mange infected wombats. n-6 acids, specifically arachidonic acid, promote a range of physiological effects, including inflammation, arrhythmia, platelet activation, and vasoconstriction (Schmitz and Ecker 2008). n-3 fatty acids can counter the effects of n-6 acids, however, a high n-6:n-3 ratio results in an inflammatory signalling response (Schmitz and Ecker 2008). Additionally, oleic acid, a fatty acid that plays a role in anti-inflammatory response, activation of immune cells, and cutaneous wound repair, decreases as mange severity increases (Cardoso *et al.* 2011, Carrillo *et al.* 2012).

Thus, our fatty acid results are indicative of functional shifts toward generalised chronic inflammatory, and inhibited anti-inflammatory, functional responses in wombats.

The fatty acid results provide another line of evidence supporting a primary immune response to *S. scabiei* of inflammation in an attempt to clear the parasite and repair tissues (Pence and Ueckermann 2002). However, prolonged inflammation without mediation has a metabolic cost, and can result in loss of tissue function (Medzhitov 2010). Skin lesions are also common signs of mange disease (Pence and Ueckermann 2002), and the inability to heal lesions makes the host prone to secondary bacterial infections (McCarthy *et al.* 2004). The imbalance in fatty acid composition in mange infected wombats likely contributes to the progression of the physiological effects of mange disease, and may accelerate host mortality. Further research into the consequences of prolonged inflammation, even after the infection has been cleared, will be critical for wildlife recovery.

This study contributes to the rich body of knowledge linking *S. scabiei* infection to the phenotype of mange disease. We establish new connections showing that: (i) mangy wombats lose a greater amount of heat to the environment, with substantial energetic cost, (ii) mange infection causes an increase in field metabolic rate that requires increased foraging activity to counter, (iii) infected wombats cannot effectively meet this increased metabolic demand through increased foraging efforts and actually increase their time spent inactive, and, (iv) mange infection results in an imbalance of fatty acids, which may feed back into the cascade of physiological impacts of disease, particularly associated with chronic inflammation. Sarcoptic mange is an emerging infectious disease that causes significant disease burden, economic impacts in animal production industries, and has raised conservation concerns in wildlife populations (Tompkins *et al.* 2015, Arlian and Morgan 2017, Old *et al.* 2018). Our research has implications for treatment and rehabilitation of mange infected individuals. For example, it may be practical to combine treatment efforts with high calorie food supplementation to efficiently combat mange infection in wild and domestic animals. Such an approach may also be possible at population scales, and may enhance the outcomes of treatment methods in the field. Further research in this area would be valuable.

I. Mange scores based on hair loss percentage.

Score	*Hair loss*
0	No signs, healthy wombat
1	Ambiguous; possible hair thinning, but not clear
2	Clear signs of hair thinning, possible skin reddening
3	Small bald patches ≤10% of area
4	Moderate bald patches 10-20%
5	20-30%
6	30-40%
7	40-50%
8	50-60%
9	60-70%
10	≥70% of body covered, very poor body condition, severe

II. Individual wombat details used for Aims I, III, and IV. Location Grid zone designation is 55G.

Aim I - Quantifying heat loss						
Wombat ID	Age	Sex	Season	Location	UTM1	UTM2
T01	J	-	Mar. 2014	Narawntapu	0466482 E	5444789 N
T02a	A	-	Mar. 2014	Narawntapu	0466482 E	5444789 N
T02b	A	-	Mar. 2014	Narawntapu	0466482 E	5444789 N
T10	A	-	Apr. 2014	Narawntapu	0466482 E	5444789 N
T14	A	-	June 2014	Narawntapu	0466482 E	5444789 N
Aim III - Resting and foraging behaviour						
Wombat ID	Age	Sex	Season	Location	UTM1	UTM2
W002	A	F	Apr. / May2015	Narawntapu	0466482 E	5444789 N
W006	A	F	Apr. / May2015	Narawntapu	0466482 E	5444789 N
W009	A	M	Apr. / May2015	Narawntapu	0466482 E	5444789 N
Aim IV - Fat composition						
Wombat ID	Age	Sex	Season	Location	UTM1	UTM2
F02	A	M	Sept. 2015	Port Arthur	0567965 E	5223099 N
F03	A	M	Dec. 2015	Forcett	-	-
F04	A	F	Dec. 2015	Anthill Ponds	-	-
F05	A	F	Apr. 2016	Broadmarsh	0510025 E	5276659 N
F08	A	M	Feb. 2016	East Derwent	-	-
F09	A	F	June 2016	Tasmania	-	-
F10	A	M	July 2015	Brighton	0520463 E	5272433 N
F11	A	F	Aug. 2015	Brighton	-	-

III. Sensible heat loss was defined as the sum of convective heat loss (free and forced) and radiative heat loss, following the methods of Cross *et al*. (2016). Free and forced convective heat losses were calculated using the following equations:

Eq. 1 $$Nu\ free = B\ Gr^m$$

Eq. 2 $$Nu\ forced = D\ Re^n$$

where Gr is the Grashof number, Re is the Renolds number, and B, m, D, and e are constants based on surface shape and orientation (Monteith and Unsworth 2013). Radiative heat loss was calculated using the equation:

Eq. 3 $$R = \frac{1}{A}\int \varepsilon\ \sigma\ \left(T_{surface}^4 - T_{surrounding}^4\right) d\ A$$

whereby A is the area of the body region, Ɛ is the emissivity of the wombat fur (assumed to be 0.95), T surface is the temperature of the wombat segment derived from the thermal imagery, and T surrounding is the ambient temperature.

IV. Breakdown of behaviours for each wombat (W002, W009, and W006) across four days.

W002 - Healthy (M=1)												
Activity	Day 1 (April 24)			Day 2 (April 27)			Day 3 (April 30)			Day 4 (May 4)		
	Total # of bouts	Avg. bout dur. (sec)	Longest bout (sec)	Total # of bouts	Avg. bout dur. (sec)	Longest bout (sec)	Total # of bouts	Avg. bout dur. (sec)	Longest bout (sec)	Total # of bouts	Avg. bout dur. (sec)	Longest bout (sec)
Inactive	489	93.28	1563	774	60.62	2175	707	60.98	2202	408	117.46	2136
Restlessness	180	11.97	102	274	15.30	438	315	14.62	315	58	12.05	81
Slow walk / graze	422	85.11	2802	657	48.98	1239	617	55.45	2133	502	70.39	1350
Steady Walk	131	11.68	81	118	15.43	108	199	12.99	78	96	11.91	75
Dig	62	3.68	9	93	3.41	9	141	3.42	12	130	3.39	9
Scratch	55	4.15	12	44	4.16	9	56	4.18	9	31	4.74	12
Run	7	4.71	9	3	4.00	6	3	5.00	6	3	4.00	6
Unknown 1	45	4.60	21	38	4.66	12	70	5.31	18	39	4.69	15
Unknown 2	49	5.88	15	66	5.59	15	102	4.88	15	49	5.33	15
Unknown 3	5	3.00	3	2	4.50	6	16	3.19	6	5	3.00	3
Unknown 4	54	3.44	9	56	3.59	9	56	3.80	12	61	3.64	6

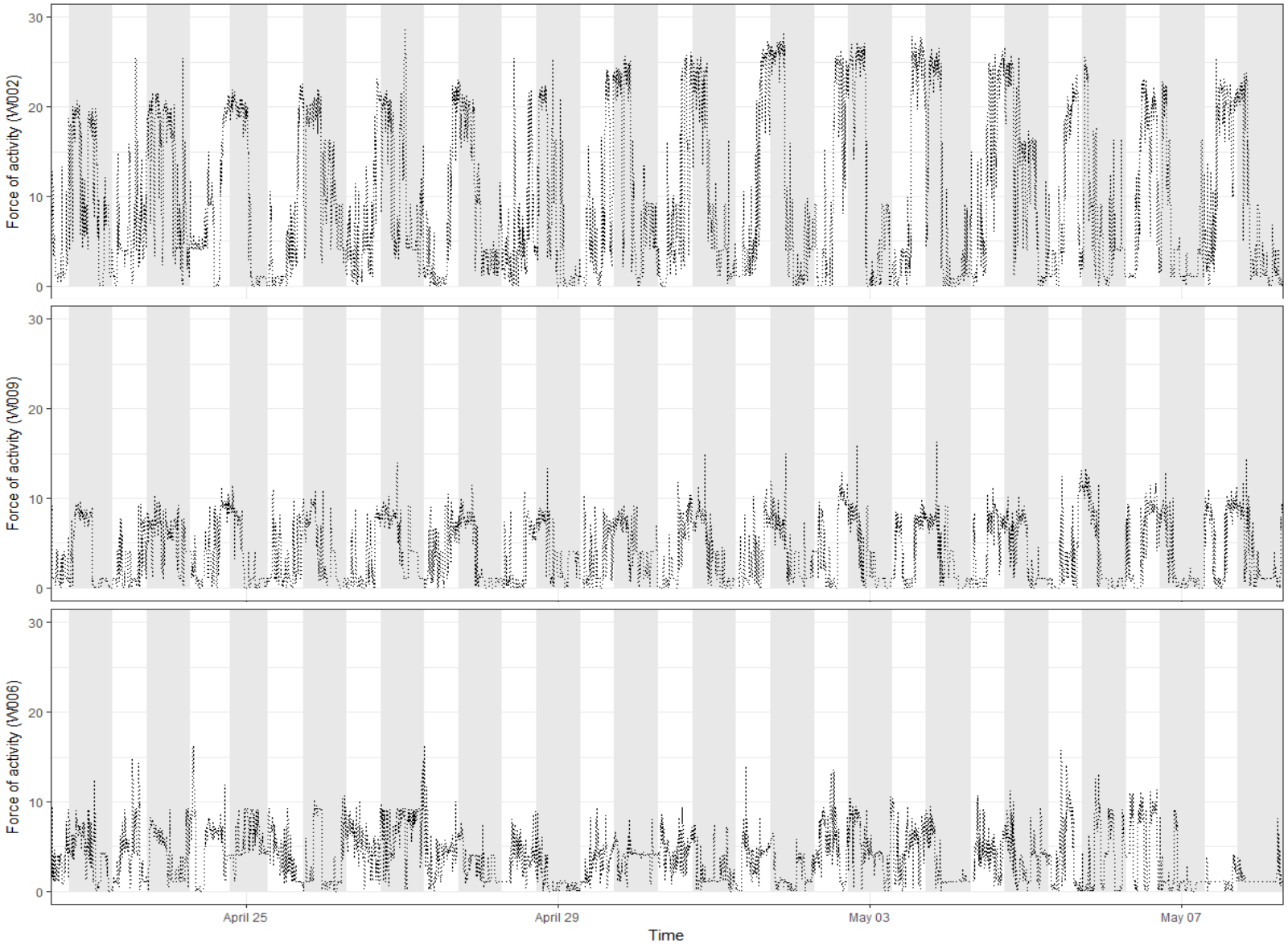
W009 – Early (Ambiguous) (M=2)												
Activity	Day 1 (April 24)			Day 2 (April 27)			Day 3 (April 30)			Day 4 (May 4)		
	Total # of bouts	Avg. bout dur. (sec)	Longest bout (sec)	Total # of bouts	Avg. bout dur. (sec)	Longest bout (sec)	Total # of bouts	Avg. bout dur. (sec)	Longest bout (sec)	Total # of bouts	Avg. bout dur. (sec)	Longest bout (sec)
Inactive	1061	44.59	2400	1087	46.79	2079	1016	50.80	3084	1161	43.12	3054
Restlessness	201	14.33	144	110	16.15	744	120	21.83	1089	54	17.11	114
Slow walk / graze	1075	30.27	516	1246	24.47	531	1245	22.66	618	1571	19.39	387
Steady Walk	180	11.60	72	141	11.09	66	129	13.50	84	168	11.59	66
Dig	184	3.24	12	270	3.90	15	384	3.90	18	531	4.53	18
Scratch	42	3.86	15	10	3.30	6	15	4.80	9	17	3.88	6
Run	15	14.00	39	5	10.80	36	0	0.00	0	2	4.50	6
Unknown 1	40	6.15	21	39	4.92	18	47	6.26	21	38	4.97	12
Unknown 2	47	5.49	18	60	4.65	12	45	6.20	18	44	5.93	18
Unknown 3	3	3.00	3	3	4.00	6	2	4.50	6	2	3.00	3
Unknown 4	26	3.69	6	20	3.90	6	15	3.40	9	14	3.86	12

W006 - Moderate (M=6)												
Activity	Day 1 (April 24)			Day 2 (April 27)			Day 3 (April 30)			Day 4 (May 4)		
	Total # of bouts	Avg. bout dur. (sec)	Longest bout (sec)	Total # of bouts	Avg. bout dur. (sec)	Longest bout (sec)	Total # of bouts	Avg. bout dur. (sec)	Longest bout (sec)	Total # of bouts	Avg. bout dur. (sec)	Longest bout (sec)
Inactive	1220	49.56	750	1821	28.68	738	1589	35.29	1191	1825	27.72	735
Restless	364	15.57	408	327	11.30	108	115	11.53	114	168	8.09	108
Slow walk / graze	652	26.12	198	1376	19.22	432	1306	17.13	225	1470	18.67	246
Steady Walk	2	22.50	36	42	14.65	45	114	19.98	90	110	21.19	81
Dig	15	4.40	12	10	4.64	9	12	3.69	12	18	5.68	12
Scratch	65	3.05	6	96	3.09	9	63	3.10	6	114	3.34	18
Run	0	0.00	0	0	0.00	0	0	0.00	0	1	6.00	6
Unknown 1	165	6.24	27	147	8.55	39	195	7.85	27	186	9.56	60
Unknown 2	186	8.53	30	173	9.05	42	273	8.46	54	235	8.85	45
Unknown 3	1	6.00	6	1	3.00	3	1	0.00	0	1	6.00	6
Unknown 4	81	3.74	12	64	3.61	6	72	3.50	6	84	3.43	9

V. Behaviours averaged across four days for each wombat.

W002 - Healthy (M=1)								
Activity	Number of Bouts (all days)			Bout Duration (sec) (all days)			Average time spent (per day)	
	Average	S.D.	S.E.	Average	S.D.	S.E.	%	S.E.
Inactive	594.5	173.96	86.98	77.20	73.30	1.50	53.12	1.20
Restless	206.75	114.14	57.07	14.09	11.01	0.38	3.37	1.06
Slow Wlk. grz.	549.5	107.43	53.72	62.62	62.11	1.32	39.83	0.95
Stdy. wlk.	136	44.41	22.21	13.09	4.26	0.18	2.06	0.35
Dig	106.5	36.08	18.04	3.47	0.42	0.02	0.43	0.07
Scratch	46.5	11.68	5.84	4.26	0.69	0.05	0.23	0.02
Run	4	2.00	1.00	4.50	0.63	0.16	0.02	0.01
Unk. 1	48	14.99	7.49	4.89	0.99	0.07	0.27	0.05
Unk. 2	66.5	24.99	12.49	5.32	0.98	0.06	0.41	0.06
Unk. 3	7	6.16	3.08	3.21	0.26	0.05	0.03	0.01
Unk. 4	56.75	2.99	1.49	3.62	0.49	0.03	0.24	0.01
W009 - Early (M=2)								
Activity	Number of Bouts (all days)			Bout Duration (sec) (all days)			Average time spent (per day)	
	Average	S.D.	S.E.	Average	S.D.	S.E.	%	S.E.
Inactive	1081.25	60.72	30.36	46.21	65.33	0.99	57.82	1.08
Restless	121.25	60.58	30.29	16.91	21.46	0.97	2.37	0.51
Slow Wlk. grz.	1284.25	207.38	103.69	23.69	13.70	0.19	35.21	1.02
Stdy. wlk.	154.5	23.56	11.78	11.94	3.84	0.15	2.13	0.13
Dig	342.25	150.15	75.07	4.07	0.70	0.02	1.61	0.45
Scratch	21	14.31	7.15	3.96	0.71	0.08	0.10	0.03
Run	5.5	6.66	3.33	11.87	3.65	0.78	0.08	0.06
Unk. 1	41	4.08	2.04	5.62	1.11	0.09	0.27	0.03
Unk. 2	49	7.44	3.72	5.49	1.07	0.08	0.31	0.01
Unk. 3	2.5	0.58	0.29	3.60	0.42	0.13	0.01	0.00
Unk. 4	18.75	5.50	2.75	3.72	0.54	0.06	0.08	0.01
W006 - Moderate (M=6)								
Activity	Number of Bouts (all days)			Bout Duration (sec) (all days)			Average time spent (per day)	
	Average	S.D.	S.E.	Average	S.D.	S.E.	%	S.E.
Inactive	1613.75	284.74	142.37	33.98	25.56	0.32	63.47	2.55
Restless	243.5	120.70	60.35	12.37	9.28	0.30	3.49	1.21
Slow Wlk. grz.	1201	372.12	186.06	19.42	8.68	0.13	27.00	2.74
Stdy. wlk.	67	54.49	27.25	19.87	5.56	0.34	1.54	0.68
Dig	13.75	3.50	1.75	4.96	0.95	0.13	0.08	0.02
Scratch	84.5	24.80	12.40	3.17	0.37	0.02	0.31	0.05
Run	0.25	0.50	0.25	1.50	1.00	1.00	0.00	0.00
Unk. 1	173.25	21.55	10.77	8.07	1.99	0.08	1.62	0.19
Unk. 2	216.75	46.03	23.02	8.70	1.79	0.06	2.18	0.21
Unk. 3	1	0.00	0.00	3.75	0.96	0.48	0.00	0.00
Unk. 4	75.25	9.07	4.53	3.57	0.45	0.03	0.31	0.02

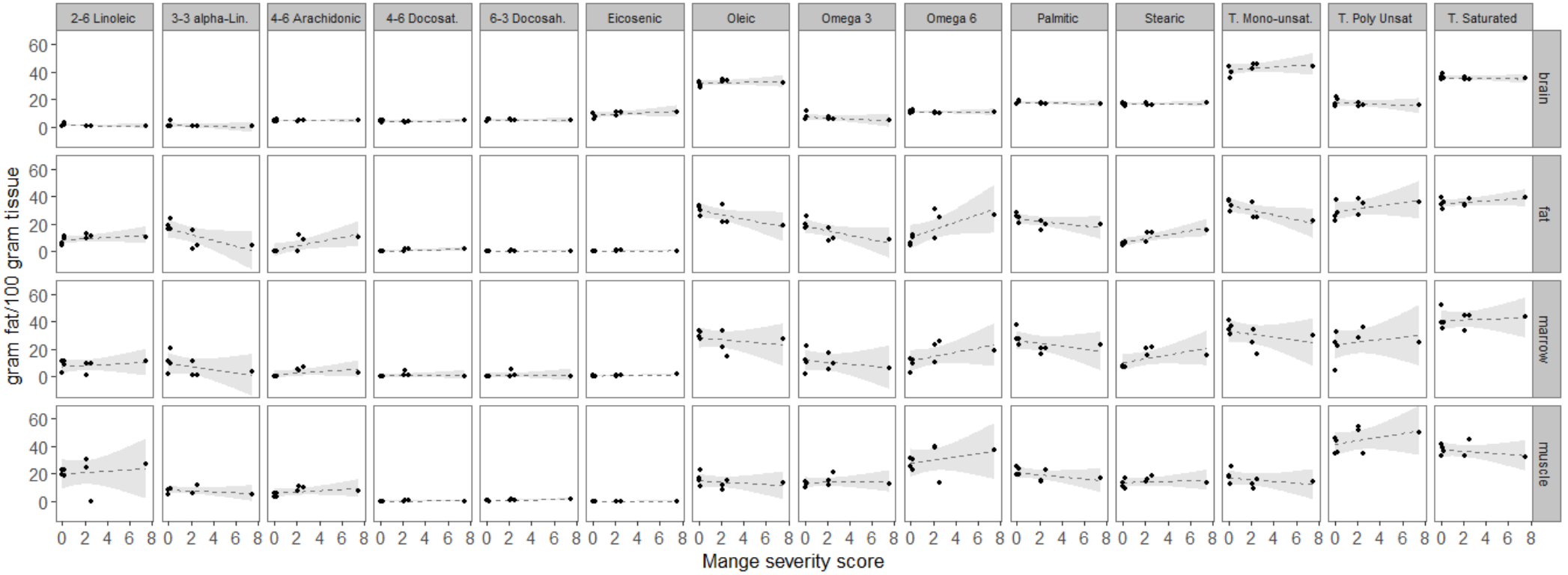
VI. Circadian behaviour and force of activity for three wombats across 16 days.



VII. Results from the screening principle component analysis to identify fatty acids with the best explanatory power.

Fatty Acid	Brain			Fat			Bone marrow			Muscle		
	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
	86.4%	7.2%	3.3%	83.8%	12.6%	2.7%	55.8%	32.7%	7.7%	74.5%	19.7%	4.1%
C4:0 Butyric	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.01	-0.01	0.00
C6:0 Caproic	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C8:0 Caprylic	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C10:0 Capric	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C12:0 Lauric	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C14:0 Myristic	-0.03	0.04	-0.01	-0.03	-0.01	-0.02	-0.05	-0.02	-0.11	0.01	0.03	0.02
C15:0 Pentadecanoic	-0.01	0.02	0.01	0.00	0.00	0.02	-0.01	-0.01	-0.03	0.00	0.00	-0.01
C16:0 Palmitic	-0.17	-0.07	0.13	-0.19	-0.20	**-0.37**	**-0.30**	-0.22	**-0.40**	0.22	0.20	-0.25
C17:0 Margaric	-0.02	0.02	0.02	-0.01	-0.01	0.00	-0.01	-0.02	-0.04	0.01	0.00	-0.02
C18:0 Stearic	0.02	**0.36**	**-0.42**	0.24	-0.10	-0.17	**0.31**	-0.19	**0.43**	0.02	**-0.34**	-0.29
C20:0 Arachidic	0.00	0.02	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.01
C22:0 Behenic	0.00	0.00	0.00	0.01	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00
C24:0 Lignoceric	-0.01	-0.06	0.01	0.02	0.00	0.02	0.00	0.00	0.01	0.00	0.00	0.00
Total saturated	-0.21	0.29	-0.27	0.04	**-0.32**	**-0.50**	-0.06	**-0.44**	-0.15	0.26	-0.13	**-0.52**
C14:1 Myristoleic	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C16:1 Palmitoleic	-0.03	0.06	0.02	-0.05	-0.02	-0.05	-0.11	-0.04	-0.13	0.02	0.05	0.03
C17:1 Heptadecenoic	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C18:1 Oleic	0.29	0.03	**0.48**	**-0.30**	-0.18	**0.47**	**-0.34**	0.16	0.29	0.16	**0.37**	**0.33**
C20:1 Eicosenic	**0.33**	0.04	-0.24	0.01	0.00	0.02	0.01	-0.02	-0.02	0.00	0.00	0.01
C22:1 Docosenoic	0.00	-0.03	-0.02	0.01	0.00	0.02	0.00	0.00	0.01	0.00	0.00	0.00
C24:1 Nervonic	0.01	0.01	0.01	0.01	-0.01	0.00	0.00	0.00	0.01	0.00	0.00	-0.01
Total monounsaturated	**0.60**	0.11	0.25	**-0.32**	-0.22	**0.46**	**-0.44**	0.10	0.16	0.19	**0.42**	**0.34**
Omega-6	-0.05	**-0.53**	-0.21	**0.55**	0.07	0.20	**0.42**	-0.17	-0.11	**-0.56**	0.04	-0.05
Omega-3	**-0.35**	0.25	**0.35**	-0.28	**0.46**	-0.14	0.07	**0.50**	-0.02	0.09	**-0.31**	**0.33**
C18:2-6 Linoleic	-0.11	0.12	0.06	0.12	0.20	0.22	0.10	-0.02	**-0.51**	**-0.51**	**0.45**	-0.26
C18:3-6 gamma Linolenic	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C18:3-3 alpha Linolenic	-0.24	**0.33**	0.13	**-0.39**	**0.47**	-0.20	-0.08	**0.51**	-0.25	0.11	-0.08	0.25
C20:2-6 Eicosadienoic	0.00	-0.03	-0.05	0.01	0.01	0.01	0.01	-0.01	0.00	0.00	0.00	0.01
C20:3-6 Eicosatrienoic	0.01	-0.02	0.01	0.08	-0.03	-0.02	0.15	-0.12	0.00	-0.01	-0.06	0.01
C20:3-3 Eicosatrienoic	-0.02	0.01	0.01	0.00	0.02	-0.01	0.01	0.01	-0.04	0.01	-0.02	0.02
C20:4-6 Arachidonic	0.02	-0.28	0.00	0.29	-0.09	0.01	0.14	-0.06	0.20	-0.04	**-0.32**	0.17
C20:5-3 Eicosapentaenoic	0.00	0.08	-0.13	0.03	-0.01	-0.01	0.04	-0.02	0.03	0.00	-0.06	-0.01
C22:2-6 Docosadienoic	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.01
C22:4-6 Docosatetraenoic	0.04	**-0.30**	-0.24	0.04	-0.02	-0.01	0.02	0.03	0.19	0.00	-0.02	0.02
C22:5-3 Docosapentaenoic	-0.01	0.03	0.01	0.06	-0.01	0.04	0.08	-0.05	0.00	-0.02	-0.11	0.05
C22:6-3 Docosahexaenoic	-0.07	-0.19	**0.32**	0.02	0.00	0.01	0.01	0.05	0.25	-0.01	-0.03	0.02
Total polyunsaturated	**-0.40**	-0.28	0.14	0.27	**0.53**	0.06	**0.49**	**0.34**	-0.13	**-0.47**	-0.27	0.28
Total monotrans	-0.02	-0.03	0.01	0.00	0.01	-0.04	0.00	0.00	-0.01	0.01	0.00	-0.06
Total polytrans	-0.01	0.01	0.00	0.00	0.00	-0.01	0.00	0.00	0.00	0.00	-0.01	-0.02

VIII. Visual representation of the 14 fatty acids with the best



Chapter 3

Chapter 3.0 – Invasive pathogen drives host population collapse: effects of a travelling wave of sarcoptic mange on bare-nosed wombats

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Author's contributions: SC, JI, and CPB conceived and designed the research; AMM, SC, JI, and CPB collected all data; AMM analysed the data; AMM, SC, CPB, and TAF interpreted results; AMM, SC, and CPB drafted the manuscript, and all authors participated in manuscript modifications.

3.1 Abstract

1. Emerging and invasive pathogens can have long-lasting impacts on susceptible wildlife populations, including localised collapse and extirpation. Management of threatening disease is of widespread interest and requires knowledge of spatiotemporal patterns of pathogen spread.

2. Theory suggests disease spread often occurs via two patterns: homogenous mixing and travelling waves. However, high resolution empirical data demonstrating localised (within population) disease spread patterns are rare.

3. This study examined the spread of sarcoptic mange (aetiological agent *Sarcoptes scabiei*) in a population of bare-nosed wombats (*Vombatus ursinus*), and investigated whether pathogen spread occurred by homogenous mixing or a travelling wave.

4. Using seven years of population surveys and four years of disease severity surveys, we show that mange was first detected in the east of a wombat population in northern Tasmania, and progressed westward as a travelling wave. Wombat mortality rates reached 100% behind the wave, with a 94% decline in overall wombat abundance within the park.

5. *Synthesis and applications.* Globally distributed pathogens may have severe impacts on susceptible host species. This is the first study to quantify population level impacts of sarcoptic mange upon bare-nosed wombats, showing a wave of mange disease which resulted in a dramatic population decline. Successful management of the spread of this and similar pathogens may hinge on the capacity to establish transmission barriers at local or between-population scales.

Keywords: Sarcoptic mange, bare-nosed wombat, disease invasion, disease wave, travelling wave, disease transmission, invasive pathogens, homogenous mixing, disease spread

3.2 Introduction

Invasive pathogens impose a major threat to wildlife conservation globally (Smith *et al.* 2009, Thompson *et al.* 2010, Tompkins *et al.* 2015). The arrival of virulent pathogens into naïve host populations can result in dramatic population declines and localized host extinctions (Smith *et al.* 2009, Thompson *et al.* 2010). Notable examples include white-nose syndrome in north American hibernating bat species (Blehert *et al.* 2009) and chytrid fungus in amphibian species (Briggs *et al.* 2010). However, few empirical studies exist documenting the spatiotemporal pattern of disease spread and host population decline during epizootics. Understanding how pathogens spread through naïve and susceptible host populations is important as it can inform strategies for disease management, such as targeted treatment or vaccination campaigns (Lange *et al.* 2012).

Travelling waves and homogenous mixing represent two alternative patterns by which pathogens are observed to invade host populations. For virulent pathogens, these patterns of pathogen spread may also manifest as patterns of host decline (e.g., Skerratt *et al.* 2007; Frick *et al*. 2010). Under homogenous mixing, the pathogen rapidly spreads following arrival with little detectable spatial or temporal structure in the pattern of population decline. In contrast, with travelling waves, the pathogen spreads as a disease front, with a spatiotemporal rate of decline. The type of pathogen or disease spread pattern observed may vary with scale, even within the same system, owing to differing host-pathogen contact rates. For example, disease spread may occur by homogenous mixing at a local scale (within a population of individuals capable of randomly mixing) where host-host or host-pathogen contact rates are high, with spread by travelling wave observable at regional scales (multiple groups of non-randomly mixing individuals scales) where contact rates are low. There are several well-documented regional scale examples for both homogenous mixing and travelling waves (Lucey *et al.* 2002, Conner and Miller 2004, Lips *et al.* 2006, Biek *et al.* 2007, LaDeau *et al.* 2007, Vredenburg *et al.* 2010, Foley *et al.* 2011), while a paucity of empirical disease spread data exists at the local scale. The lack of information regarding disease spread at local scales may be due to the challenges in acquiring the high resolution data needed to reveal spread patterns. While regional patterns of disease spread are often well understood, these patterns may not be conserved, or applied, at local scales.

Research into the dynamics of pathogen spread is critical for disease management in wildlife populations particularly at local scales, because effective intervention strategies are reliant upon some understanding of the nature by which target pathogens spread (Wobeser 2002). Effective management methods for pathogen spread by homogenous mixing may include widespread vaccination events (Killian *et al.* 2007). Conversely, successful intervention for pathogens that spread via travelling waves may require alternative measures, such as establishing barriers to pathogen movement (a tactic proposed to protect livestock from wildlife disease spill-over (Gortazar *et al.* 2015). There is an urgent need to couple empirical studies of pathogen spread with advances in disease management, particularly due to the increased threat of invasive pathogens for the persistence of small, isolated wildlife populations (Smith *et al.* 2009, Tompkins *et al.* 2015).

Sarcoptes scabiei is among the most widespread of parasitic mites, infecting >100 mammal species globally (Bornstein *et al.* 2001, Pence and Ueckermann 2002). It is considered an emerging and invasive pathogen of many wildlife populations (Bornstein *et al.* 2001, Pence and Ueckermann 2002, Tompkins *et al.* 2015). The *S. scabiei* mite burrows into the outer epidermal layers of its host, causing a range of symptoms including alopecia, pruritus, hyperkeratosis, and emaciation (Pence and Ueckermann 2002). The global dispersal of this pathogen is likely the result of human host movement, with spill-over into naïve domestic animals and wildlife populations where it can have dramatic population impacts (Pence and Ueckermann 2002, Fraser *et al.* 2016). Notable examples of this pathogen impacting wildlife include wombats (Skerratt 2005), coyotes (Murray *et al.* 2015), arctic and red foxes (Mörner 1992, Forchhammer and Asferg 2000), lions and cheetahs (Gakuya *et al.* 2012), gray wolves (Jimenez *et al.* 2010), and mountain gorillas (Graczyk *et al.* 2001). Despite the array of host species affected, the spatial structure of disease spread is not well understood (Pence and Ueckermann 2002), in part due to the complexity of transmission. Transmission can occur through both direct and indirect contact, as mites can survive in the environment for short periods (Arlian *et al.* 1989). Little empirical data exists describing the pattern of mange spread during outbreaks.

Sarcoptic mange is arguably the most important disease of wombats in Australia, affecting two of the three extant species (Martin *et al.* 1998, Hartley and English 2005, Skerratt 2005, Thompson *et al.* 2009). Anecdotal reports of mange causing population declines and localised

extirpation events exist (Martin *et al.* 1998), but empirical documentation of such events are currently lacking. This knowledge gap is increasingly relevant for attempts to manage sarcoptic mange in this iconic Australian marsupial. A greater understanding of pathogen spread will help implement intervention programs for this and similar host-invasive pathogen scenarios. Using seven years of population surveys and four years of pathogen severity surveys from northern Tasmania, this study examines the spatiotemporal characteristics of pathogen spread and host population decline at a local (within population) scale. We investigate whether *S. scabiei* spread occurs via homogenous mixing or a travelling wave at this scale.

3.3 Methods

3.3.1 Ecology and social organisation of bare-nosed wombats and mange transmission

Bare-nosed wombats are the largest burrowing herbivorous mammals, and utilise a network of core and peripheral burrows to take shelter during mostly diurnal conditions (Johnson 1998b, Triggs 2009). They are solitary (having limited direct physical interaction among adult individuals) and non-territorial (Evans 2008, Favreau *et al.* 2010). Sarcoptic mange infections in wombats cause severe alopecia, skin lesions, and parakeratosis (Skerratt 2003b). Wombats may transmit mange between one another by direct contact or via the environment, with environmental transmission being the more widely supported hypothesis (Skerratt *et al.* 1998). Potential environmental transmission may occur through burrow sharing.

3.3.2 Data collection

3.3.2.1 Field location and transect surveys

This study took place at Narawntapu National Park (NNP), located on the central-north coast of Tasmania, Australia (-41.15°N, 146.60°W, see Figure 3.1). The park contains a variety of habitats, including costal heathlands, eucalypt and she oak forest, grassland, schlerophyll woodland, bracken/scrubland, and ex-agricultural land. The portion of the park that was historically converted to grassland for pasture (3.29 km^2) and used as farmland is now utilized by macropods and wombats for grazing. These areas are surrounded on three sides: the east

by a small mountain range (Briggs Regional Reserve), the west by Port Sorrell, and the north by the Bass Strait. These natural features create a semi-isolated wombat population, with the only potential avenues for immigration/emigration being through the south and east (Figure 3.1). Open landscapes and the increased diurnal behaviour of Tasmanian wombats (relative to mainland wombats, see Discussion) allow for extensive monitoring of the population at NNP, and thus a unique opportunity to document spatial and temporal changes in wombat abundance and disease spread.

Wombat abundance surveys were conducted annually from 2010, which coincided with the year a mange outbreak was first detected in this population. These abundance surveys were later supplemented by disease severity surveys, conducted simultaneously from 2013. Combined, these surveys document the spatiotemporal impact of *S. scabiei* on the wombat population, and spread of the pathogen, as assessed by visual signs of mange. For wombat abundance, 12–15 transects were walked each September within the grassland habitat of the park. All transects were walked at dawn, simultaneously, and repeated once per day for 3–4 consecutive days (effort 2010–12, 2016 = 3 days; 2013–15 = 4 days). Observations of each wombat included the distance from the transect (using a Nikon Forestry Pro Range Finder) and the transect number. Natural geographic features within NNP result in five logical areas for which transects and observations of wombats occur within, but not between (Figure 3.2). These features are predominately tree lines along drainage ditches, but also include buildings between Areas 3 and 4. Within each area, there was some minor variation in the number and placement of transects each year (see Supplementary Material I). Thus, wombat abundance was estimated for each park area, based on total kilometres of transect surveyed within a given area, and averaged across survey days (3–4 days) to produce the average number of wombats per kilometre. These surveys enabled documentation of spatiotemporal wombat decline during the mange outbreak.

Targeted mange severity surveys were performed from 2013–2016. Two groups of observers started in opposite ends of the park (one in the east, one in the west), surveying the entirety of each area by walking systematic north-south sweeps, and converging at approximately the central area of the park. As surveying groups came into visual contact, communication via hand-held radio was used to ensure all wombats were only recorded once. For each wombat spotted, a GPS location and mange severity score were taken. These surveys started at noon

and continued until all wombats in the grasslands were scored. Wombats were observed using a Leica TELEVID 77 spotting scope (20–60x zoom) and Nikon binoculars (10x24), and infections were visually diagnosed.

Mange scores were assigned following Simpson *et al*. (2016), by which the wombat is divided into 14 body segments and each is assigned a mange score from 0–10. Clinical signs of mange that are visually obvious develop around 14 days after infection (Skerratt 2003b). Our surveys were based on visual observation of clinical mange, but the actual disease front is likely ahead of the visual front. Thus, the front reported here is indicative of symptomatic disease. We ranked mange severity by the highest body segment score assigned to an individual wombat, as follows: highest segment score of 0–2 healthy, 3 early mange, 4–6 moderate mange, 7–8 severe mange, 9–10 late stage (see Supplementary Material II). Individuals with body segment scores ≥9 are less common due to mortality from poor body condition, emaciation, and infection severity that accompany this state. We believe, based on longitudinal observations, that this is a slightly more robust approach than our previous methodology (Simpson *et al.* 2016) where a segment score of two was indicative of mange. Analyses performed with the Simpson *et al*. (2016) classification deviated only slightly from the results presented here (see Supplementary Material III).

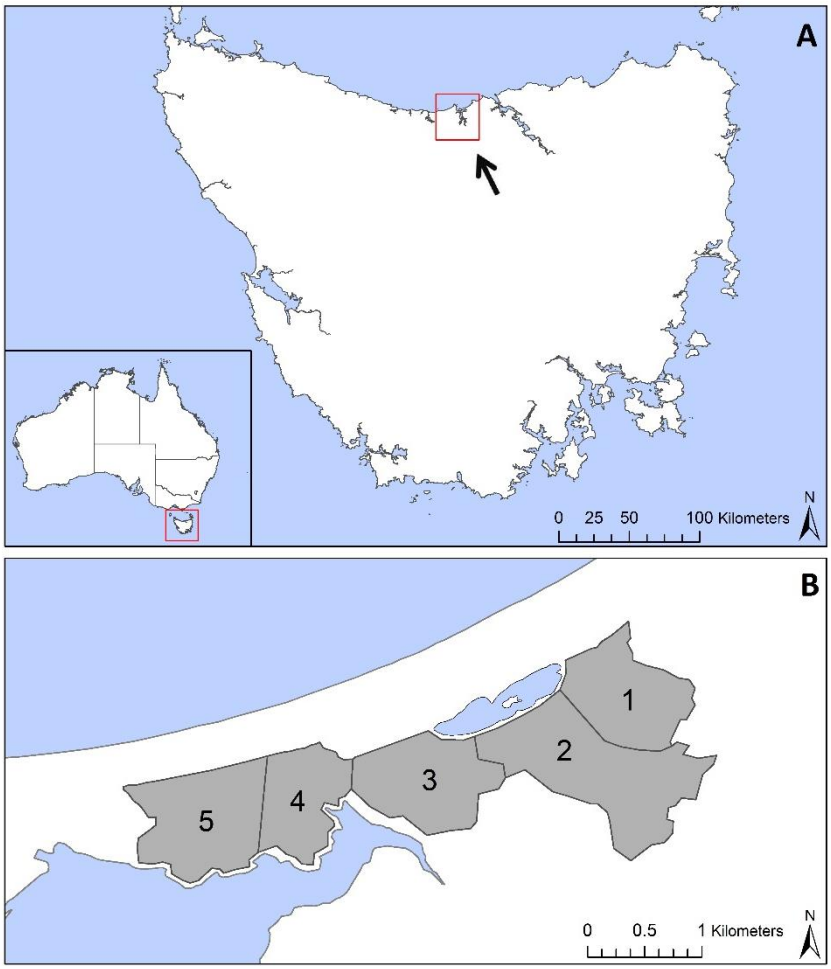


Figure 3.1. A) Narawntapu National Park, located on the central-north coast of Tasmania, Australia (-41.15°N, 146.60°W). B) The five surveying areas within Narawntapu National Park (grey shading). The total surveying area is 3.29 km^2. Areas are numbered from east to west, corresponding with the first observation of mange (originated in Area 1). The areas are pasture, which are surrounded by forest, waterways, and hills.

3.3.3 Analyses

3.3.3.1 Wombat abundance and spatial decline

We used the multi-year wombat abundance surveys to test the spatial and temporal pattern of population decline in the park. To understand the park-wide change in wombat abundance from 2010–2016 (mean number of wombats per km, across all transects), a linear regression was performed. To explore temporal patterns in changing wombat abundance within each area, piece-wise linear models were used. Each area was fitted with two linear segments (segmented R package; Muggeo 2008) to identify a) when wombat abundance began to decline, and b) the intensity of the decline (slopes of the segments). To understand the spatiotemporal component of declining wombat abundance, the relationship between the distance of each area from the eastern grazing limit and the midpoint (year) of respective area decline was examined using a linear regression. The outbreak is confirmed to have begun in the east, but the exact location of the origin in the eastern area is not certain. Therefore, the distance to each area was quantified as the distance (in meters) from the eastern grazing limit of the park (longitude 146.62°W) to the longitudinal midpoint of each area.

3.3.3.2 Mange front progression and connecting disease to wombat abundance

To understand shifts in the geographic distribution of mange and estimate the rate of pathogen movement within NNP 2013–2014, 2014–2015, and 2015-2016, wombat locations and mange severity scores were mapped using ArcMap 10.2. The surface between wombat locations was smoothed using inverse distance weighting (IDW) interpolation based on the mange score assigned. The mange front progression was defined by the longitudinal change of the western-most location of an individual with one or more body segments exhibiting a score ≥3.

To assess the population effect of sarcoptic mange on wombats, we evaluated the relationship between mange prevalence and wombat abundance. This relationship was quantified for NNP Areas 3–5, although Area 3 was excluded from the statistical analyses owing to only having a single year of mange and wombat abundance data before this area was extirpated. Mange prevalence calculations were based on the number of wombats in the area with any body segment scoring ≥3, with 95% confidence intervals calculated using the 1-sample proportions

test with continuity correction (program R; Newcombe 1998). We used a Bayesian mixed effects regression model, with park area as a random effect, to test how mange prevalence predicted wombat abundance (MCMCglmm R package; Hadfield 2010). We also demonstrated that the relationship obtained was not due to park area differences by conducting linear regression analyses for each area, independently. Analyses for each area were conducted from the year before mange was observed to year 2016.

3.4 Results

3.4.1 Wombat abundance and spatial decline

Wombat abundance at NNP declined significantly from 2010–2016, dropping 93.6% from 18.6 wombats/km in 2010 to 1.2 wombats/km in 2016 (linear regression, R^2=0.7215, $F_{1,22}$=60.57, *P* <0.001; Figure 3.2). The decline in wombat abundance was observed in all five park areas, and complete collapse was observed in three of five areas by 2016 (abundance = 0, Figure 3.3). The mange outbreak started in the eastern end of the park with significant declines initiating in 2010 for Area 1 (*P* <0.001), Area 2 (*P* <0.001), and Area 3 (*P*=0.002), based on the piece-wise linear regressions (Table 3.1; piece-wise linear regression *P*-values represent slope coefficients, significant values correspond to slopes that are significantly different from zero). These areas experienced complete population collapse in 2014, 2016, and 2014, respectively. Rates of decline varied within the eastern and central areas, with the steepest declines occurring in Area 2, followed by Area 1, and more gradual decline in Area 3 (Table 3.1). Continued progression of mange through the western areas of the park resulted in a wave-like decline, with Area 4 experiencing significant declines starting in July of 2013 (breakpoint estimate= 2013.56; decline *P*= 0.005), and Area 5 declines beginning in April of 2014 (breakpoint estimate= 2014.38; decline *P*= 0.184). There was a notable relationship between the distance of the areas from the eastern end of the park and the year that abundance declines began within areas (R^2= 0.76, $F_{1,3}$=13.83, *P*=0.03; Figure 3.4), showing a spatiotemporal wave of decline.

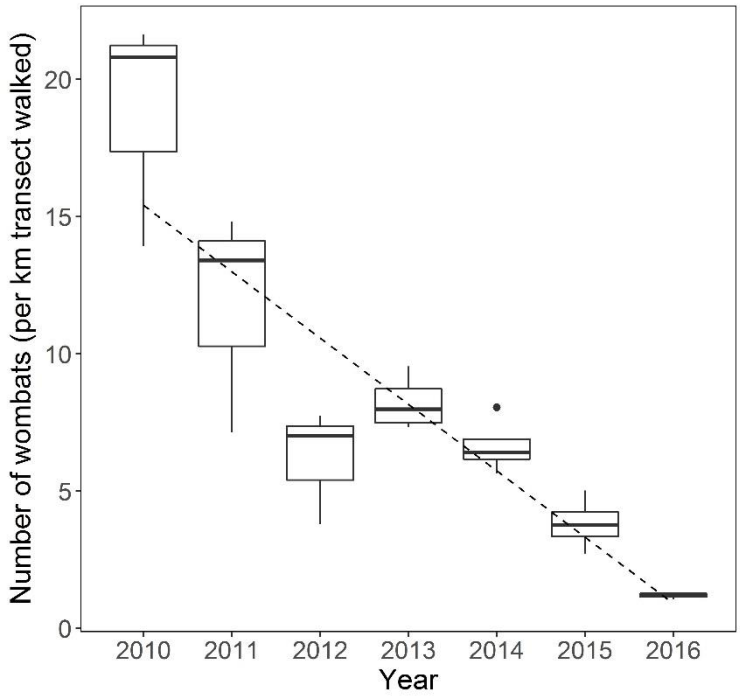


Figure 3.2. The change in wombat abundance at Narawntapu National Park from 2010 to 2016 (R^2=0.7215, $F_{1,22}$=60.57, *P* <0.001). Wombat abundance was derived from all transects surveyed in all areas, and was averaged across 3–4 sampling days for each year. The median number of wombats is presented by the black line within each box and the linear regression is the dotted line. Over six years, wombat abundance declined by 93.6%.

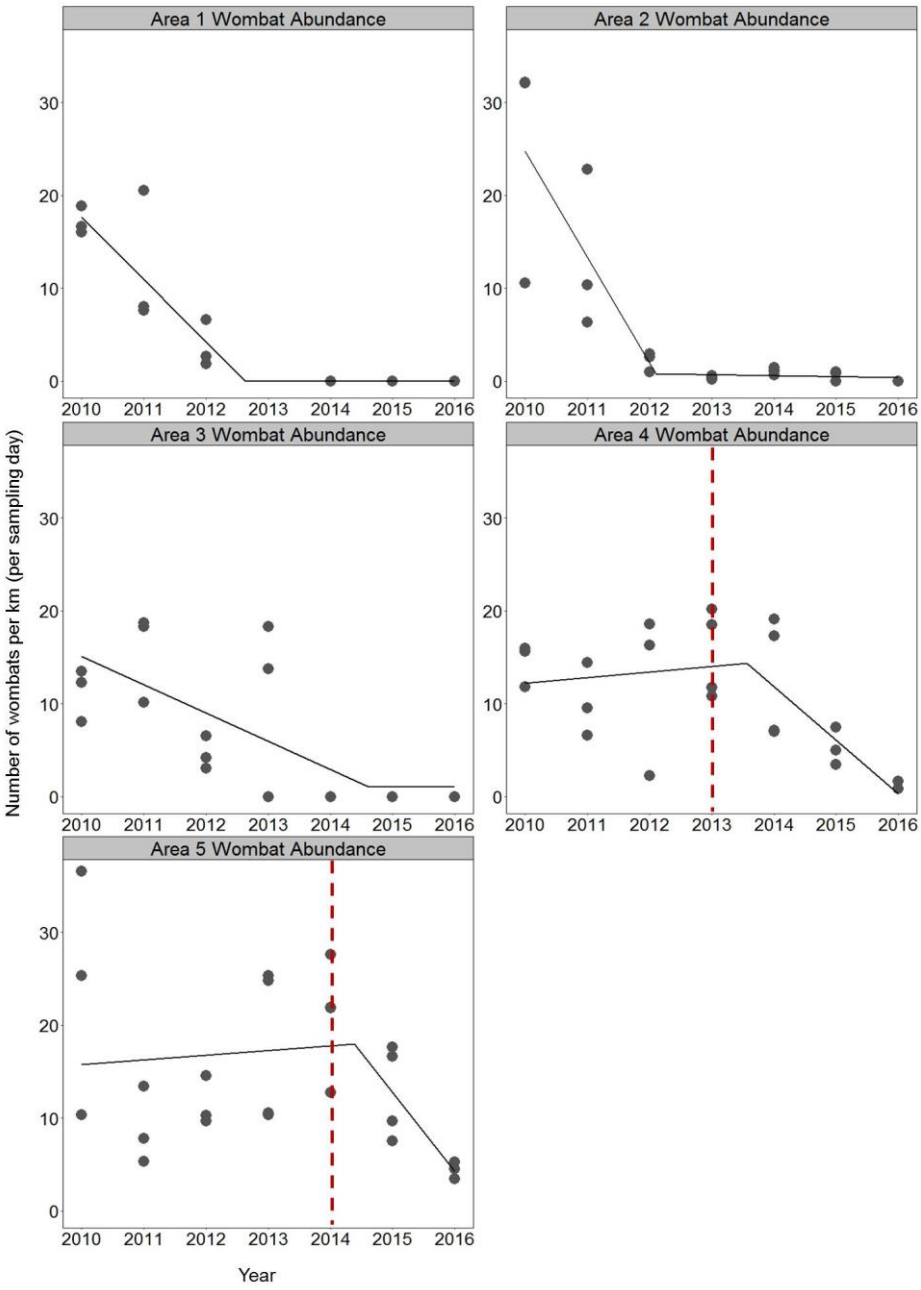


Figure 3.3. Temporal and spatial decline of wombats within Narawntapu National Park. Each area of the park (Figure 3.1B) was surveyed from 2010–16 (effort 2010–12, 2016 = 3 days, 2013–15 = 4 days). Piecewise linear models were used to quantify the relationship between wombat abundance and time. Each linear model was fitted with two segmented relationships. The eastern end of the park was the location where the outbreak was first observed in 2010, and Areas 1, 2, and 3 exhibited declines from 2010. Consecutive declines were observed as the front progressed into the west: Area 4 (2013) and Area 5 (2014). The vertical, red dashed line in Areas 4 and 5 represent the arrival of the mange front.

Table 3.1. Patterns of wombat abundance over time for each area of Narawntapu National Park. Patterns assessed by piecewise linear regressions, fitted with segmented relationships (two per area).

Area	Regression piece 1			Breakpoint		Regression piece 2			R-Squared
	Coefficient	S.E.	P-value	Estimate	S.E.	Coefficient	S.E.	P-value	(overall)
1	-6.73	± 1.61	<0.001	2012.63	± 0.50	< 0.01	± 1.08	1.00	0.85
2	-11.38	± 1.96	<0.001	2012.10	± 0.35	-0.16	± 1.15	0.893	0.76
3	-3.04	± 0.86	0.002	2014.62	± 1.45	< 0.01	± 3.86	1.00	0.47
4	0.60	± 1.61	0.611	2013.56	± 0.61	-5.74	± 1.83	0.005	0.44
5	0.51	± 1.37	0.715	2014.38	± 0.89	-8.45	± 6.15	0.184	0.13

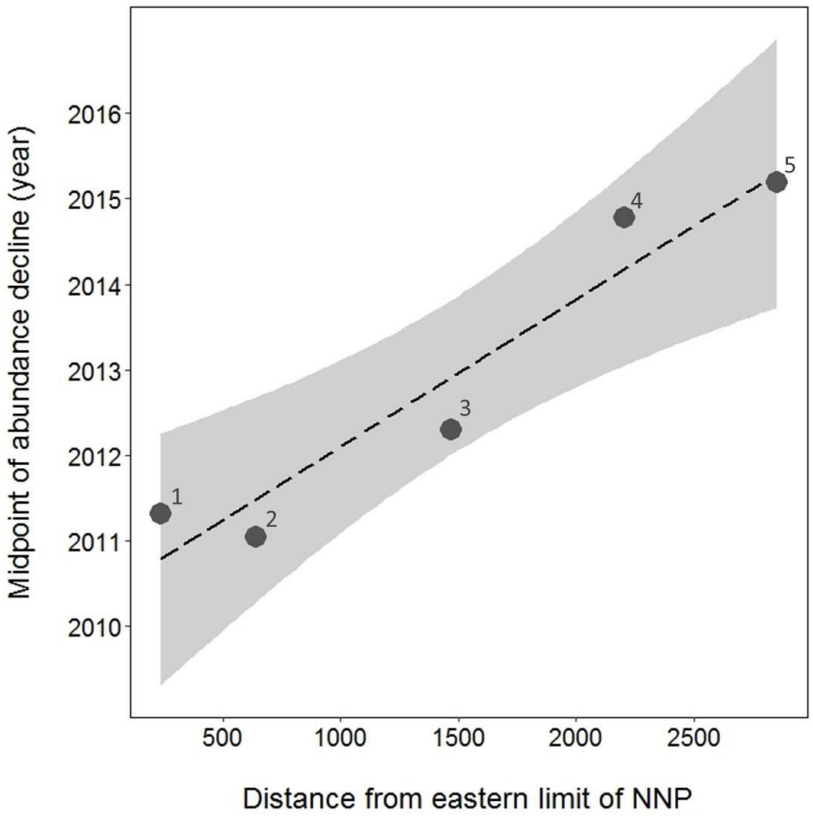


Figure 3.4. The spatiotemporal pattern of wombat decline within Narawntapu National Park as derived from piecewise linear regression estimates of the midpoint of the decline slope within each area. There is a strong correlation between the distance an area (numbered 1-5 on graph) is from the eastern limit of the park, and the midpoint (year) of the decline in that respective area (R^2= 0.89, $F_{1,3}$=34.39, P<0.01).

3.4.2 Mange front progression and prevalence-abundance relationships

A total of 105 wombats were scored for mange across four years. Each received either a full score (all 14 segments, N=98), a half score (entire right or left side, N=6), or a partial score (<7 segments of one side, N=1). Half and partial scores were the result of wombats fleeing to burrows before score completion. The mange front progressed westward annually from 2013–16 (Figure 3.5). The western limit of mange infections varied between years, moving 733 m west between 2013 and 2014, an additional 276 m west between 2014 and 2015, and only 6 m between 2015 and 2016 as a consequence of reaching the western most limit of the parks observable wombat foraging area. The net progression was 1015 m over four consecutive survey years (average per year 338.3m ±367.5 S.D.).

Decline in wombat abundance within areas correlated with the movement of the mange front into these areas. As mange prevalence within a given area increased, wombat abundance decreased (Figure 3.6). Mange entered Area 4 in 2013 (S. Carver, personal observation) and Area 5 in 2014. A significant negative relationship between mange prevalence and wombat abundance was observed (coefficient= -14.977; 95% CI: -26.53, -3.87; *P* =0.018; Figure 3.7), where wombat abundance decreased as mange prevalence increased. Linear regressions revealed this relationship was consistent within areas (Area 4 abundance, 2013-2016, β=-0.19 ±0.03 S.E., R^2= 0.946, $F_{1,2}$=53.93, *P*=0.02; Area 5 abundance, 2014-2016, β=-0.12 ±0.001 S.E., R^2= 0.999, $F_{1,2}$=9159, *P*<0.01), showing western wombat population declines occurring concomitantly with the westward spread of sarcoptic mange.

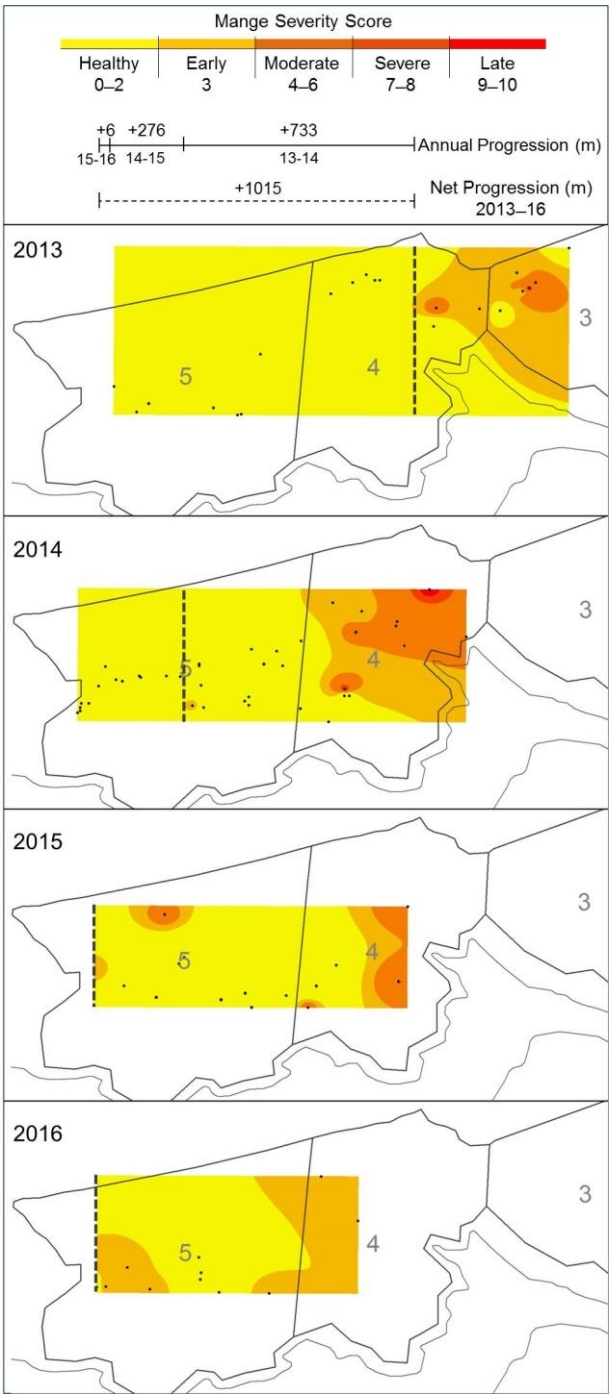


Figure 3.5. Progression of mange across Narawntapu National Park. Four consecutive years (2013–16) of wombat locations with accompanying mange scores plotted in ArcMap, smoothed with inverse distance weighting (IDW) interpolation. Each black point represents an individual wombat observed. The mange front (dotted black line) progressed westward each year, moving a total of 1015 meters from 2013–16. The size of the mange severity box represents the extent of wombats observed (black dots). Areas of NNP (Figure 3.1) are denoted by grey numbers.

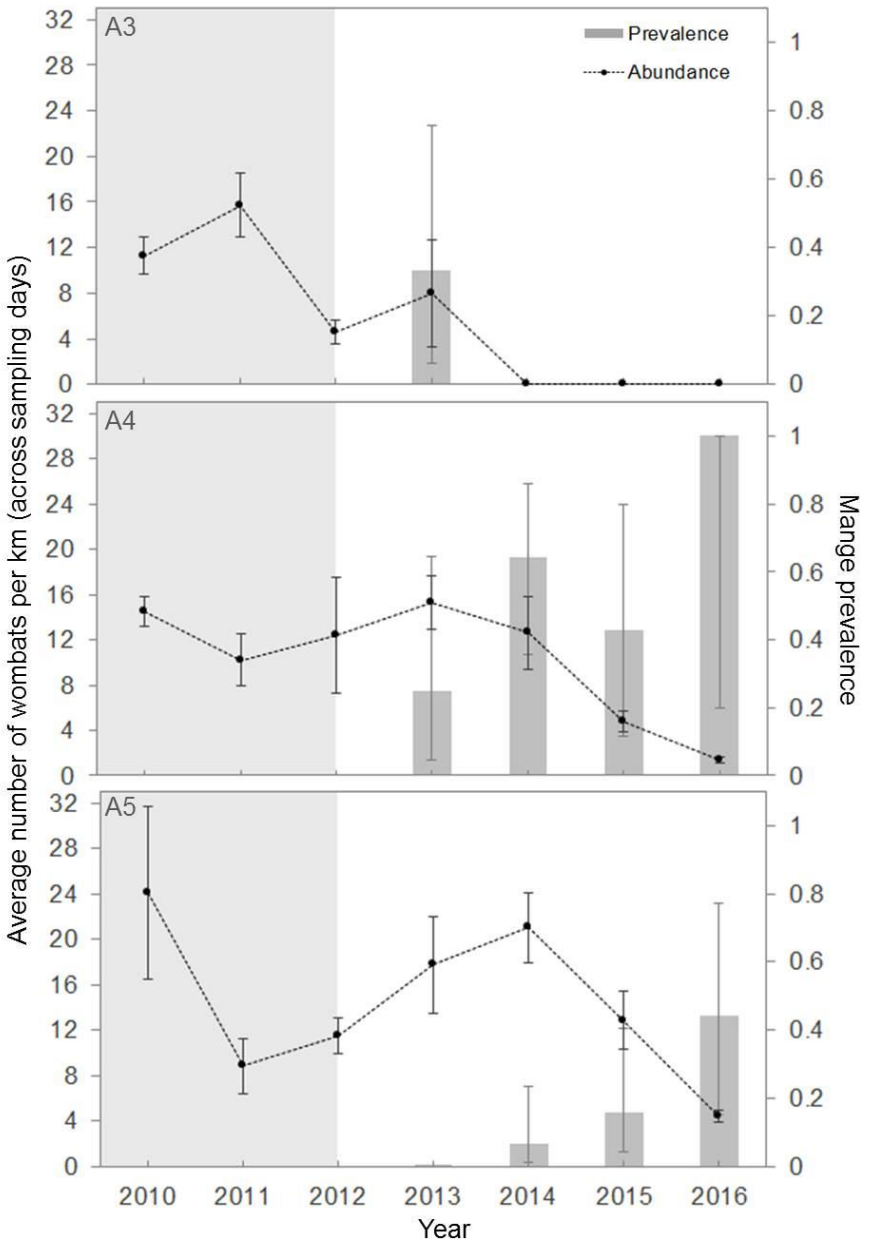


Figure 3.6. Mean (±S.E.) change in wombat abundance and mange prevalence (± 95% C.I.) over time. Graphs are based on the four consecutive years (2013–16) in which combined abundance and mange data were collected for Areas 3, 4, and 5 (A3, A4, and A5). Mange prevalence is quantified as the proportion of wombats exhibiting visual signs of mange. Collection of prevalence data began in 2013 (non-shaded part of graph), and thus there is uncertainty as to when mange entered Area 3. Mange entered Area 4 in 2013 and Area 5 in 2014. See Figure 3.7 for the relationship between wombat abundance and mange prevalence.

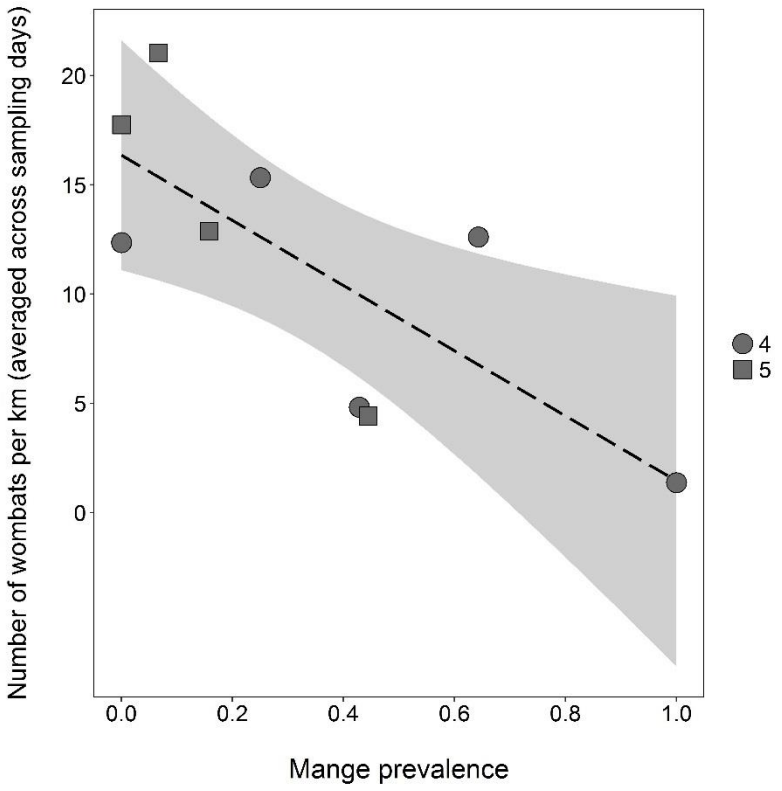


Figure 3.7. The relationship between wombat abundance and mange prevalence within Narawntapu National Park Areas 4 and 5. The relationship was assessed using abundance data from Areas 4 and 5, including the year prior to mange arrival (prevalence = 0) through to the last year of data collection (2016). As mange prevalence increased, wombat abundance decreased (Bayesian mixed effects regression with park area as the random effect – coefficient= -14.977; 95% CI: -26.53, -3.87; *P* =0.018). Linear regressions were run for both Areas 4 and 5, using only years with both prevalence and abundance data (Area 4 2013-2016, Area 5 2014-2016). Regressions revealed significant abundance declines for both areas after the arrival of mange (Area 4, R^2= 0.946, $F_{1,2}$=53.93, *P*=0.02; Area 5, R^2= 0.999, $F_{1,2}$=9159, *P*<0.01).

3.5 Discussion

Through high spatial resolution surveys, we show that sarcoptic mange disease spreads as a travelling wave through a population of bare-nosed wombats. Invasive pathogens have the capacity to significantly reduce the population size of naïve hosts, and potentially drive localized extinction (Daszak *et al.* 2000, Lafferty and Kuris 2005, Pedersen *et al.* 2007). This pathogen caused a substantive population impact, resulting in a 100% decline of wombats in the eastern and central areas of the park, with a 94% decline overall and may lead to population collapse in the near future. This study is the first to empirically demonstrate the impact this pathogen can have on a wombat population. Further, this study contributes empirical information on pathogen spread at the local (within population) scale, which, to the best of our knowledge, is rare in the literature.

Disease spread information is pivotal for developing management and intervention strategies. There remains much to be learned about how mange impacts wombats at a national level. Nevertheless, our study has some clear implications for mange disease management in threatened wombat populations. Our research suggests that effective management of mange may be achieved by establishing barriers (either physical or immunological) to pathogen spread across populations, or where feasible, by administration of a population scale treatment. Research testing management strategies of *S. scabiei* in wombat populations is currently underway (Chapter 4, in review). Owing to the global distribution of this pathogen, these findings may also apply to other impacted wildlife species, and more broadly to other similarly transmitted pathogens (Cunningham *et al.* 2008, Foley *et al.* 2016).

This study is the first to empirically document mange impacts upon a bare-nosed wombat population. There have been anecdotal reports of wombat population collapse in response to the disease previously (Martin *et al.* 1998), but empirical documentation has until now remained elusive. Reports of sarcoptic mange outbreaks date back to the 1930's (Gray 1937, Skerratt *et al.* 1998). The often nocturnal behaviour of wombats has perhaps led to a dearth of population and pathogen studies for these marsupials. The combination of our results and anecdotal studies may indicate a significant disease burden upon bare-nosed wombats (possibly also southern-hairy nosed wombats) at a national scale, which should be investigated further. However, few reports have historically considered mange to be a mechanism for the

extirpation of stable populations, as the disease spread is assumed to be self-limiting, based on host density (Pence and Ueckermann 2002). Our study of an ongoing epizootic that is projected to result in localized extinction challenges this assumption. Mange may persist and impact wombats across a range of dynamic scenarios, from epizootic to stable chronic infectivity (Martin *et al.* 1998, Skerratt *et al.* 2004b, Ruykys *et al.* 2009, Ruykys *et al.* 2013), and we suggest that more critical quantitative assessments of the impacts of sarcoptic mange are necessary.

More broadly, this would not be the first instance of sarcoptic mange driving isolated and/or small host populations to extinction. Two examples include the Bornholm island red fox population in Denmark (Henriksen *et al.* 1993) and the isolated Las Rasos Spanish ibex population in Spain (León-Vizcaíno *et al.* 1999). In the first example, a mange epizootic extirpated the naïve fox population from Bornholm. In the latter example, a prior mange epizootic isolated the Las Rasos ibex population within Cazorla National Park. The disease persisted in the ibex population, only to flare again, driving the isolated population to extinction within five years. These examples, as well as our study, showcase the vulnerability of isolated and/or small populations to mange outbreaks. Two studies of mange spread at regional scales show a wave of spread (Soulsbury *et al.* 2007, Turchetto *et al.* 2014); however, ours is the first study to document the pattern of pathogen spread (including rates of advancement) at a within population scale, providing information which may be vital for disease mitigation. Mitigation of sarcoptic mange is particularly important in populations that are subjected to additional threats, such as the critically endangered northern hairy-nosed wombat (Hartley and English 2005).

Predictions about the spatiotemporal spread of pathogens and associated management efforts are likely heavily influenced by host social behaviour. This is particularly the case when infection impacts host behaviour, which can change disease spread pattern. Sarcoptic mange infections result in a range of behavioural changes in the host which potentially impact the pattern of disease spread (Chronert *et al.* 2007, Soulsbury *et al.* 2007). Specifically, behavioural changes for wombats affected by mange include traveling further than healthy wombats (seasonally, Skerratt *et al.* 2004), increased diurnal activity (Ruykys *et al.* 2009, Borchard *et al.* 2012), spending more time scratching and drinking (Simpson *et al.* 2016), and spending more time being active outside of the burrow (Simpson *et al.* 2016). These changes may be the result

of host manipulation by the pathogen to enhance transmission opportunities. This is particularly plausible when behavioural changes result in visitation to a greater number of burrows, given the hypothesized role that mite deposition in the burrow plays in transmission (Skerratt *et al.* 1998, Skerratt *et al.* 2004b). However, the specific contribution of these behavioural changes on mange transmission dynamics is yet to be fully explored. Understanding behavioural changes and the projected progression of disease provides an opportunity for management to act ahead of the disease front, protecting uninfected and susceptible individuals.

It is possible that diurnal activity of mangy wombats, compared to more nocturnal behaviour of healthy wombats (Borchard *et al.* 2012), influenced detection of wombats in the eastern and central areas, confounding our conclusions of host population collapse in these areas. False signals of collapse could have resulted from the die-out of diurnally active, mangy wombats while nocturnally active, healthy wombats went undetected. However, two lines of evidence suggest this is not the case. (1) We informally undertook visual inspections of these areas each year, intensively searching for wombat scat, diggings, and burrow activity, all of which could indicate false-negative conclusions of host collapse. These inspections re-affirmed our conclusions and suggest wombats are not colonising these eastern most areas of NNP from the west or through immigration. (2) Our surveys from within the same population show Tasmanian wombats are more likely to be diurnal, regardless of health status (Simpson *et al.* 2016), as a result of less restrictive thermal conditions (Triggs 2009, Hogan *et al.* 2011).

Disease is one of several threatening processes acting on bare-nosed wombat populations. Other major threats include: a reduced and fragmented range since European settlement (McIlroy 1995, Buchan and Goldney 1998); increased negative human interactions (Ramp *et al.* 2005); predation by feral species (Banks 1997); and other disease threats (Skerratt 1998, Donahoe *et al.* 2015). Here, we exemplify the need to understand the impacts of mange, especially on a formerly stable host population. While mange itself impacts host populations, the combination of threatening processes may exacerbate disease driven effects. For example, increased fragmentation creates a risk for isolated populations affected by mange, such as in this study, to be locally extirpated. A highly infectious disease, such as mange, has a high likelihood of affecting spatially separated, fragmented, or distinct populations due to its longevity within populations (Cross *et al.* 2005). Further, the loss of isolated populations

throughout the bare-nosed wombat range may deplete genetic diversity and reduce gene exchange among metapopulations (Frankham 2005).

Continued research into variables affecting mange transmission pattern is required to understand the complexity of mange epizootics. Specifically, there is a critical knowledge gap regarding the role that indirect (environmental) transmission plays in disease spread. Mange can be spread through direct contact, but also has an environmental component whereby under certain abiotic conditions, the *S. scabiei* mite can survive off a host for several weeks (Arlian *et al.* 1989, Pence and Ueckermann 2002). Burrows provide a suitable microclimate for the *S. scabiei* mite, and likely act as a transmission pathway through burrow sharing by wombats (Skerratt *et al.* 1998). This environmental pathway hypothesis currently lacks scientific support, but remains a plausible hypothesis for more formal investigation. Further research into the frequency and mechanisms driving burrow sharing among wombats, the role of environmental pathogen spread and persistence, and disease dynamics of sarcoptic mange in other wombat populations are needed to fully appreciate the impacts of this pathogen across Australia. Filling these critical knowledge gaps will allow for more focused management and mitigation efforts.

5.0 Conclusions

Here we utilized empirical data to explore two hypotheses of pathogen spread within host populations, showing that a travelling wave model was supported for the invasion of sarcoptic mange into a bare-nosed wombat population. This pathogen had profound impacts upon the host population causing 100% mortality as the wave progressed. Our study contributes empirical evidence of the rate sarcoptic mange has spread in a bare-nosed wombat population. The evidence of spatiotemporal patterns for disease progression may also apply to other host populations, depending on host behaviour and density.

While this study helps to fill the knowledge gap regarding the effect and management of mange in Australian wildlife, there is still a need for further research. Critical questions remaining include: 1) what role do other Australian fauna (native and non-native) play in the spread of the mange mite? 2) what role does the burrow play in transmission and in mite

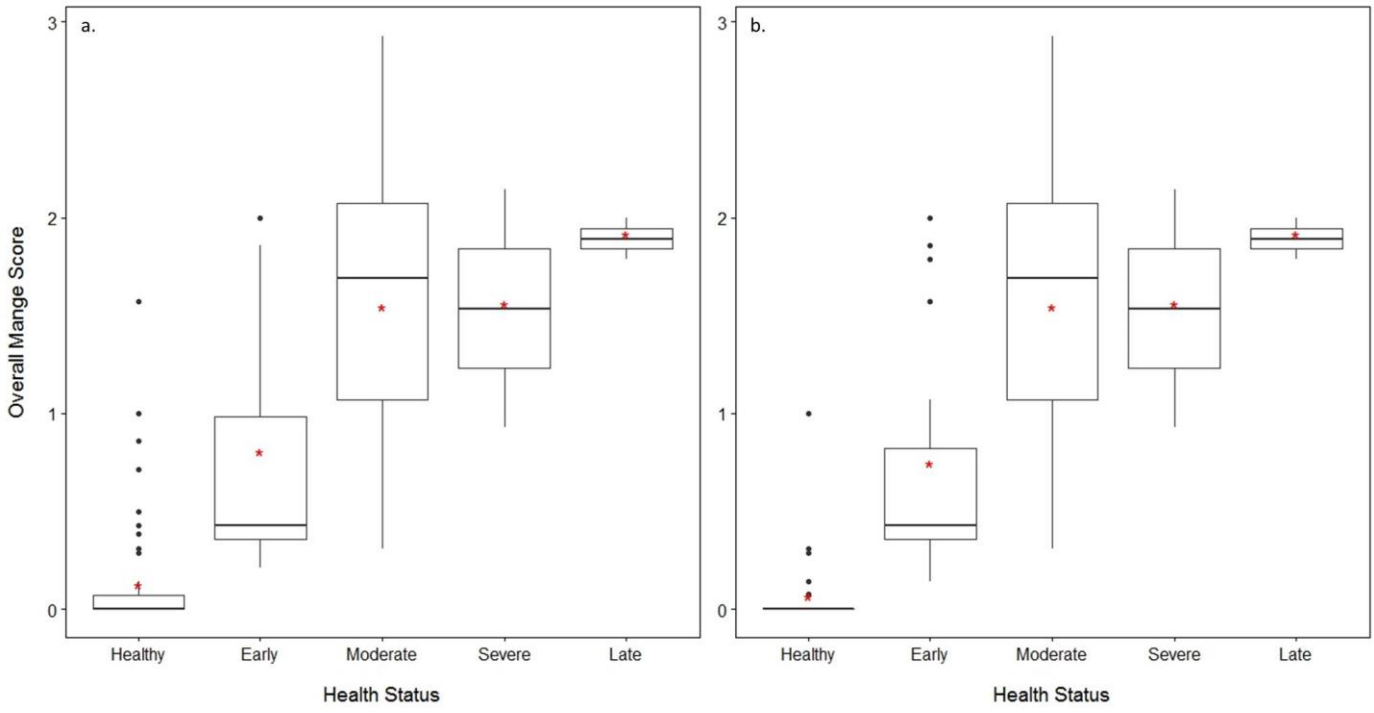
survival? 3) what are the full effects of mange on host behaviour and physiology, and can these be managed (food supplementation, barrier to movement, etc.)? 4) are similar declines/spread patterns occurring in other wombat populations at a national scale? Sarcoptic mange is among the most globally widespread of wildlife emerging infectious diseases (Tompkins *et al.* 2015), but is nonetheless a treatable infection. Through advances in understanding pathogen spread (such as in the present study), disease dynamic modelling, and future experiments into field treatment strategies, there are opportunities to establish evidence-based strategies of disease control for this and similarly transmitted pathogens in threatened wildlife populations.

Supplementary Material – Chapter 3

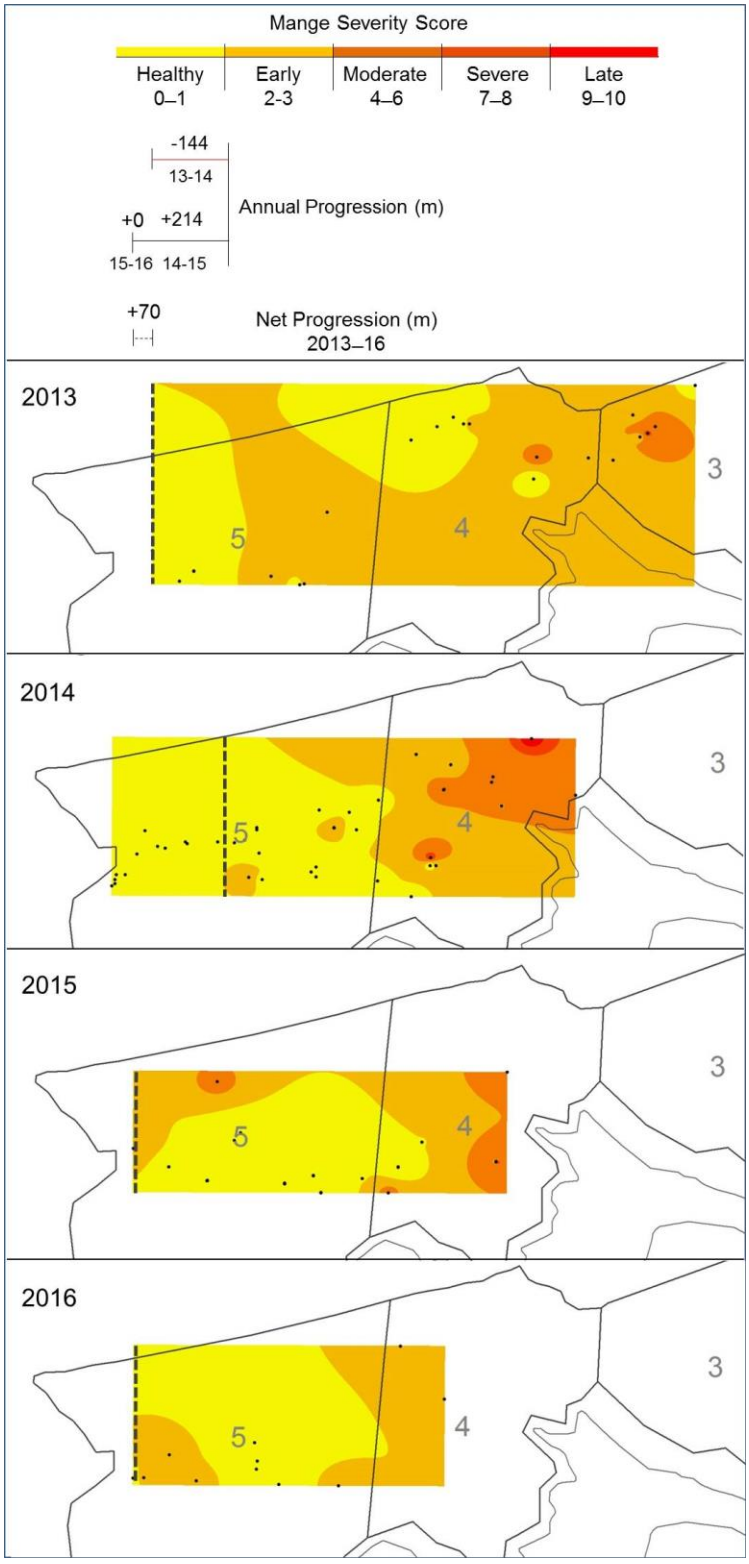
I. The number of transects walked each survey year in Narawntapu National Park, with breakdown by park Area.

Area	Number of transects walked						
	2010	2011	2012	2013	2014	2015	2016
1	2	3	2	X	2	2	2
2	4	4	3	6	3	3	3
3	2	3	2	2	2	2	2
4	4	3	3	3	3	3	3
5	3	2	3	3	2	2	2
Total	15	15	13	14	12	12	12

II. Overall mange scores were calculated using, (a) a conservative mange diagnostic, and (b) a sensitive mange diagnostic. For the conservative diagnostic, a mange infection is considered present in any individual with a body segment with a score of ≥3. With the sensitive mange diagnostic, a mange infection is considered present in any individual with a body segment with a score of ≥2 (consistent with Simpson *et al*. 2016). The conservative mange diagnostic is more consistent with our field observations, and was therefore utilized in this paper (by which a wombat with a highest segment score of 0–2 is healthy, 3 has early mange, 4–6 has moderate mange, 7–8 has severe mange, and 9–10 is in a late stage mange infection).



III. The progression of mange across Narawntapu National Park was analysed using the sensitive mange diagnostic (Supplementary Material II). The front moved westward a net total of 70 m from 2013–16.



Chapter 4

Chapter 4.0 – Population-scale treatment informs solutions for control of environmentally transmitted wildlife disease

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Author's contributions: SC, AMM, SAR, and CPB conceived and designed the research; AMM, SC, AP, TAF and CPB collected the data; AMM, SC, and SAR analysed the data; SAR developed the statistical models, AMM, SC, SAR, CPB, and AP interpreted results; AMM, SC, and SAR drafted the manuscript, and all authors participated in manuscript modifications.

4.1 Abstract

Long-term pathogen control or eradication in wildlife is rare and represents a major challenge in conservation. Control is particularly difficult for environmentally transmitted pathogens, including some of the most conservation-critical wildlife diseases. We undertook a treatment program aimed at population-scale eradication of the environmentally transmitted *Sarcoptes scabiei* mite (causative agent of sarcoptic mange) during an epizootic in bare-nosed wombats (*Vombatus ursinus*). Field trial results were used to parameterize a mechanistic host-disease model that explicitly described indirect-transmission, host behaviour, and viable disease intervention methods. Model analysis shows that elimination of *S. scabiei* in the wild is most sensitive to the success of treatment delivery, and duration of the program. In addition, we found the frequency that wombats switch burrows was an important positive driver of mite persistence. This research emphasises the utility of applying model-guided management techniques in order to achieve practical solutions in the field. Our approach and findings have applicability to other species affected by *S. scabiei* (e.g., wolves, red foxes, Spanish ibex, and American black bear), as well as other conservation-critical systems involving environmental transmission (e.g., bat white-nose syndrome and amphibian chytridiomycosis).

Key words: Bare-nosed wombat; environmental transmission; host-disease modelling; sarcoptic mange; Sarcoptes scabiei; Vombatus ursinus; wildlife disease management

4.2 Introduction

The control of infectious disease in wildlife populations remains among the most challenging frontiers in disease ecology and epidemiology (Joseph *et al.* 2013). While much disease control theory focuses on direct or vector transmitted pathogens (Keeling and Danon 2009), some of the most significant contemporary pathogens of global conservation concern involve an environmental transmission component. Notable examples include chytridiomycosis in amphibians (Kilpatrick *et al.* 2010), white-nose syndrome in bats (Blehert *et al.* 2009), chronic wasting disease in corvids (Gilch *et al.* 2011), and anthrax in ungulates (Hassim *et al.* 2017) – all of which have resulted in host declines at regional and/or global scales. Control of environmentally transmissible pathogens must reduce or eliminate pathogens on both the host and in the environment, or more difficultly, induce resistance in the host. Pathogen control on the host and in the environment has been achieved where pathogen reservoirs are limited and influx of diseased hosts is minimal (Bosch *et al.* 2015). However, practical advances in the management of environmentally transmitted pathogens, outside of this limited context, remain a problem.

The microscopic, burrowing mite, *Sarcoptes scabiei*, causes significant disease to wild and domestic animals (mange), and humans (scabies) (Walton and Currie 2007, Tompkins *et al.* 2015). This host generalist has been documented to affect >100 mammal species and, in humans, causes 300 million cases of scabies globally (Arlian and Morgan 2017). *S. scabiei* is among the 50 most prevalent diseases of humans, and has recently been labelled a Neglected Tropical Disease (NTD) by the World Health Organization (Hay *et al.* 2014). It is responsible for epizootics in domestic and wild animals, including localised extinction events (Pence and Ueckermann 2002). This mite can be killed by a range of parasiticides, including moxidectin, ivermectin, fluralaner, and permethrin (Hay *et al.* 2012, Arlian and Morgan 2017). Treatment regimes of both human and animal populations are generally successful in reducing disease prevalence (Leone 2007, Gakuya *et al.* 2012), but often fail to eradicate the mite outside of highly controlled scenarios (see León-Vizcaíno *et al.* 2001, Menzano *et al.* 2007). Population-scale management of *S. scabiei* in wildlife is largely limited by the poorly understood environmental components of transmission (Arlian *et al.* 1988, Arlian *et al.* 1989), variable host immunity to the parasite influencing reinfection (Little *et al.* 1998, Bhat *et al.* 2017), and the

risk of mite resistance with prolonged or widespread parasiticide usage (Mounsey and McCarthy 2013).

In Australia *S. scabiei* (thought to be introduced approximately 200 years ago with European settlers and their animals; Fraser *et al.* 2016), is now endemic in aboriginal communities with prevalence rates as high as 50% (Hay *et al.* 2012). It has established itself in several native and invasive animal species (Pence and Ueckermann 2002), causing substantive ethical, animal welfare, and conservation concern. The most impacted wildlife hosts are wombats (two of the three extant species are affected), with the bare-nosed wombat (*Vombatus ursinus*, also known as the common wombat) experiencing the most severe morbidity and mortality (Skerratt 2005). *S. scabiei* occurs throughout the range of the bare-nosed wombat (Martin *et al.* 1998). Enzootic transmission is punctuated by occasional mange epizootics that significantly reduce wombat abundance (Martin *et al.* 1998, Skerratt *et al.* 1998), as well as drive small or isolated populations to the brink of extinction (Martin *et al.* 2018a). The mite can survive and remain infectious off of the host in humid and cool environmental conditions (for up to 19 days) (Arlian *et al.* 1984a, Arlian *et al.* 1989). Wombat burrows, which are cool and humid relative to the ambient environment, are thought to act as an environmental reservoir for disease transmission and reinfection in this solitary species. Successful treatment of individual infected wombats has been achieved through the use of ivermectin or moxidectin (Skerratt 2003b, Skerratt *et al.* 2004b, Ruykys *et al.* 2013); however, no population-scale attempt at disease control has been documented. Evidence for wombat recovery without intervention is equivocal, and population outbreak research shows recovery of infected individuals is unlikely during epizootics (Martin *et al.* 2018a).

Here, we test the practicality of controlling environmentally transmitted parasitic disease. We present the outcome of a treatment program aimed at eradicating *S. scabiei* from a local bare-nosed wombat population in Tasmania, Australia. We administered topical moxidectin (Cydectin®) to wombats via the use of remote treatment dosing stations ('burrow flaps', Figure 4.1A and 4.1B), and replenished the stations weekly for 12 weeks. In order to understand how this treatment program likely impacted mite prevalence, we developed two mechanistic models. The first model used observations of dosing station triggering to estimate the rate wombats moved between burrows. This important rate parameter was then incorporated into a novel host-disease model that tracked the transfer of mites between wombats and their

burrows. Next, we estimated delivery success of each dose application during flap triggering and the transfer rates of mites between wombats and their burrows, by comparing observed levels of mange prevalence with the model's predictions. Sensitivity analyses were then performed to predict how modifications to the treatment program (e.g., program duration and burrow coverage), and the duration of protection conferred to hosts, would likely impact mite eradication in the field. The essential elements of our study system and model (i.e. environmental transmission of disease, temporary protection of hosts against infectious agents, and host use of retreat sites) are common to a number of species impacted by *S. scabiei* worldwide, as well as other wild host-pathogen systems.

4.3 Methods

4.3.1 Study system

Bare-nosed wombats are large (15-30 kg), burrowing herbivores that utilise a network of core and supplementary burrows (using 4-12 burrows on average; Taylor 1993, Skerratt *et al.* 2004a, Evans 2008) for rest and refuge (Johnson 1998b, Triggs 2009). Wombats are largely solitary and non-territorial, with contact among adults limited generally to mating (Evans 2008, Favreau *et al.* 2010); they often exploit the same burrows, but usually asynchronously (Skerratt *et al.* 2004a, Walker *et al.* 2006). Though mange may be transmitted among wombats by direct contact, environmental transmission via bedding chambers within burrows is the more widely supported hypothesis and also evidenced by empirical patterns of pathogen spread (Skerratt *et al.* 1998, Martin *et al.* 2018a). Accordingly, intra-specific transmission of *S. scabiei* likely occurs through asynchronous sharing of burrows among individuals. Other host species can be involved in *S. scabiei* transmission, but the absence of canids at our study site supports pathogen persistence via a single host species.

4.3.2 Sarcoptic mange treatment trial

Our population-scale treatment regime of sarcoptic mange took place at Narawntapu National Park (NNP; Tasmania, Australia, 0466482 E, 5444789 N; Supplementary Material). A mange outbreak began at NNP in 2010, spreading from east to west across the park killing 100% of the bare-nosed wombats behind the disease front, and depleting the bare-nosed wombat abundance by >90% (Martin *et al.* 2018a). During 2015, management efforts based on existing knowledge regarding wombat behaviour, mite biology, and drug properties (Table 4.1), were concentrated in a 1.1 km^2 area of the park where wombats remained. Moxidectin and ivermectin have been used to successfully and safely treat mange infections in wombats (Skerratt 2003b, Death *et al.* 2011, Ruykys *et al.* 2013) and confer protection (0.2 mg kg-1 dose of moxidectin lasts for an average of 5 days within a wombat; Death *et al.* 2011), with several methods by which these treatments can be delivered (e.g., topically, orally, and intramuscularly). For *in-situ* administration, topical treatment via remote dosing stations (e.g., burrow flaps) has been employed as a non-invasive and recommended method for pathogen control in individuals (2017b). Burrow flaps comprise a frame secured over the wombat burrow entrance with a hinged door (flap) hanging down from the frame that deposits treatment to wombats, exiting or entering, via a small treatment reservoir (Figure 4.1A and 4.1B). Suggested treatment frequency for topical application is one dose (1 mL 10 kg^{-1} moxidection; Cydectin®) per week for 8 weeks, followed by four, fortnightly treatments (2017b), spanning four months in entirety.

Prior to commencing the treatment experiment, all wombat burrows within the area were surveyed, mapped, and their use (active or inactive) was assessed (e.g., presence of fresh scats near entrance, cleared vegetation around burrow, fresh excavations). Of the 646 burrow entrances surveyed, 141 were deemed active, with an additional 105 considered potentially active (likely used once per fortnight) (Supplementary Materials). Surveys for wombat presence and activity (scats and diggings) support that burrows outside of the managed area were inactive. The park itself is semi-isolated (to the east by a small mountain range, to the west by Port Sorrell, and to the north by Bass Strait), with limited potential avenues for immigration/emigration from the south. Burrows were re-surveyed each week to monitor changes in activity states, and additional burrow flaps were deployed as needed (maximum of 203 installed). Burrow flaps were installed on active burrows within the managed park area,

and each was filled with 5 mL of Cydectin (our field work has found adult wombats weighed 14–26 kg at this site), ensuring a minimum of 1 mL 10 kg^{-1} dose could be achieved in all cases. Overdose (due to high single dose or multiple doses) did not pose significant risk owing to well-established wide safety margins of moxidectin (Paul *et al.* 2000, Virbac 2017). The flaps were refilled weekly for 12 weeks (an even more conservative treatment regime than suggested; DPIPWE 2017b) from 15 September 2015 to 02 December 2015 in an attempt to ensure mites die in the environment before they can reinfect treated wombats. The total number of burrows equipped with flaps each week ranged from 180–203, based on signs of burrow activity and changes in burrow availability due to seasonal flooding. Flap activation (flap position change from vertical to horizontal) was documented weekly for each burrow as an indicator of wombat use and treatment delivery.

4.3.3 Disease surveys

To monitor disease among wombats and the efficacy of the mange treatment over time, observational surveys were conducted in the evening, starting three hours prior to dusk and concluding at last light (see also Martin *et al.* 2018a). Surveys were performed weekly during the treatment regime and in the weeks following (weeks 0–14), with decreasing frequency after week 14 (fortnightly from week 16–18, monthly from week 21–65, and final surveys in weeks 93 and 103). Each week had 1–2 evenings of survey effort, either on consecutive evenings or one day apart. To avoid overestimation of wombat abundance and mange prevalence, analyses used population data from the first survey day from each week, as this consistently surveyed the majority of observed individuals (observation patterns were similar across both evenings). Wombats were observed using a Leica TELEVID 77 spotting scope (20–60x zoom), and infections were visually diagnosed. Each wombat was scored for mange using methods from Simpson *et al*. (Simpson *et al.* 2016), and mange severity was categorized based on the method presented in Martin *et al*. (Martin *et al.* 2018a). It is important to note that mange infected wombats exhibit increased diurnal behaviour (Borchard *et al.* 2012). While Tasmanian wombats (both mangy and healthy) are more likely to be active during the day relative to their mainland Australian counterparts, the number of healthy wombats are likely underrepresented in these pre-dusk surveys (DPIPWE 2017a).

A subset (n=25) of individuals were trapped and equipped with wireless identification ear tags (WID, by Wild Spy Pty Ltd, between April – June 2015, see Supplementary Material for trapping details), which allowed for individual identification across survey weeks (Figure 1D). Owing to the severity of the epizootic, high mortality was occurring during the treatment intervention. Nevertheless, we were able to reliably identify 10 individuals repeatedly during and following the treatment period, facilitating capacity to document individual health trajectories.

4.3.4 Burrow switching model

Knowing the rate at which wombats move between burrows is critical for predicting treatment success, as burrow switching provides opportunities for mites to spread within the environment and encounter susceptible hosts. The daily probability of burrow switching for our system, denoted *p*, is unknown so we first estimated it by fitting a spatially-implicit model of wombat movement to our weekly flap triggering data. Estimating a daily probability from weekly observations involved developing a hidden-state Markov model, where the hidden state is whether or not a burrow is active on a given day (i.e., occupied by a wombat the evening prior). Burrow switching moves a burrow to the inactive state. Our model also estimated the daily probability that an inactive burrow became active, which allowed us to infer the proportion of active burrows on a given day. Bayesian MCMC methods were then used to estimate *p* and provide a credible interval. We assumed a wide uniform prior when estimating *p*, however, given the large number of burrow flaps sampled, the prior had little influence on our estimate. Full details of this movement model and the statistical fitting procedure can be found in Supplementary Material.

4.3.5 Host-disease model

We modelled mange-wombat dynamics at NNP using a state-based model. Disease-host interactions, wombat movement behaviour, and implementation of the treatment program, could best be described by tracking daily changes in the state of burrows. Each evening, the state of each burrow was defined by three variables: (1) occupation by a wombat that may or may not be infected, (2) occupation by mites, and (3) equipped with an un-triggered flap

containing a viable dose of the treatment. All wombats were associated with a single burrow each evening and wombats could be in one of four mange disease states: susceptible (S), treated and immune (I), early infection (low mite densities and difficult to diagnose by observation (Fraser *et al.* 2018b); L), and established infection (high mite densities and clinical signs observable; H). Thus, there are 20 possible burrow states derived from wombat occupancy status (unoccupied, S, I, L or H), mite occupancy (present or not), and flap treatment status (flap is present and set with viable treatment, or not). Each day burrow states may change due to the following events: wombat death; unoccupied and infected burrows lose disease; infected wombats progress from low to high mite infestation; treated wombats lose their protected status; treated burrows lose their treatment viability; fresh viable treatment is applied to the burrow flaps; wombats forage and switch burrows; and wombat offspring gain independence. Mite transfer between burrows and wombats occurs primarily when wombats switch burrows. Model parameters needed to describe these 8 events and their best estimates based on the literature, where available, are presented in Table 4.1. The equations that describe the probability of each possible state-transition for each event is necessarily cumbersome and are provided in the Supplementary Information.

Three model parameters are largely unknown for our system: the probability treatment is successfully transferred to a wombat when the flap is triggered (z_S), the probability a highly infected wombat establishes a new mite population in a burrow each evening (q_H), and the probability an infected burrow results in a susceptible wombat becoming infected during an evening visit (q_B). There is also high uncertainty associated with the duration that a mite colony can survive in an inactive burrow (f, Table 4.1). We visually identified three parameter scenarios that spanned parameter space whereby the time-series of predicted burrow occupancy levels were consistent with the number of wombats observed at NNP.

Next, we used the host-disease model to predict the outcome of future, realistic adaptations to the treatment program. Specifically, we assessed improvements in treatment success (i.e., reducing disease prevalence among burrows to very low levels) if (1) success in treatment delivery, z_S, was improved, (2) the protection duration of a single treatment dose, w, was increased, (3) the duration of weekly treatments was extended, and (4) a greater proportion of burrows were treated. Model sensitivity was assessed by predicting the outcomes of these potential treatment adaptations for each of the three parameter-scenarios.

Table 4.1. *A priori*, experimentally and statistically derived values used to parameterize the state-based model for mange disease spread in bare-nosed wombats. Parameter symbols correspond to their use in the text and Supplementary Material.

Parameter	Literature and field trial values	Model value	Symbol
Wombat birth rate	Biennial[1]	547.5 days	T
Wombat life expectancy	15–25 years[2]	5475 days	L
Wombat life expectancy at low infection	-	5475 days	L_L
Wombat life expectancy at high infection	Approximately 90 days (field observations)	90 days	L_H
Low-high infection duration	35 days to first signs of alopecia[3]	30 days	d
Wombat burrow sharing	Generally, rare; in high densities[4], asynchronously[5, 6]	None	-
Burrow switching frequency	4 days[4, 6]	0.13 day^{-1}	p
Mite survival in environment[†]	5–19 days[7, 8]	10-20 days	f
Moxidectin longevity in host (half-life)	5 days (2–9.5 days)[9]	5 days	w
Treatment frequency	Weekly[10]	1 per 7 days	-
Treatment duration[‡]	Suggested 12 treatments[10]	77 days	T
Burrow treatment coverage	>50% of active burrows	0.90 day^{-1}	v
Photodegradation of moxidectin[§]	Concentration reduction (94% to 22%[11])	0.50 loss day^{-1}	p_v

[†]At 75-95% relative humidity and 10 ˚C; [‡]weekly treatments for 8 weeks, then four fortnightly treatments; [§]after 12 hour exposure of aqueous moxidectin to light. References: [1]Hogan et al. 2013, [2]Triggs 2009, [3]Skerratt 2003, [4]Skerratt et al 2004a, [5]Taylor 1993, [6]Evans 2008, [7]Arlian et al. 1984a, [8]Arlian et al. 1984b, [9]Death et al. 2011, [10]DPIPWE 2017b, [11]Awasthi et al. 2013

4.4 Results

The proportion of activated stations (burrow flaps) over the course of a week was lowest (21.6%) at week 0, and ranged from 47–67% over weeks 1–11 (with an average of 54.9% overall). At the individual burrow level, activation across treatment weeks ranged from 0-100% (Figure 4.1C). We were able to follow 10 wombats during and subsequent to the treatment regime. Individual recovery was largely successful following treatment, with 100% of the identifiable wombats at "recovering" or "healthy" states between weeks 11 and 14 – the final survey weeks when treatment effects were expected to be most visible (Figure 4.1D). The proportion of wombats showing signs of mange at NNP was 39% in the first week of the treatment regime (week 0, 14 Sept. 2015; 7 infected of 18 observed). By week 11 (30 Nov. 2015), the last week of treatment, mange prevalence had dropped to 25% (3 infected of 12 observed), and by week 18 no animals showed signs of mange (0 infected of 13 observed); however, evidence of mange in following weeks suggest some diseased individuals were present and not observed in week 18 (Figure 4.1E).

Despite the short-term success, the mange treatment regime was unsuccessful in eradicating the disease from the wombat population at NNP. While observable mange prevalence reached a low of 0% in week 18, prevalence slowly and consistently increased in the following weeks. At one year post-commencement of treatment, the wombat population had decreased and mange prevalence had exceeded that of week 0, rising to 50% (3 infected of 6 observed), with infection rates reaching as high as 86% in following weeks (6 infected of 7 observed; week 56).

Individual recovery was also unsuccessful in the long-term. The first observations of a previously recovering or healthy wombat regressing to a mange state occurred at week 16, and the last signs of wombat recovery were recorded at week 21 (grey shaded area; Figure 4.1D and 4.1E). No wombats were observed within the entirety of NNP two years post treatment. As natural geographic features isolate NNP we expect that changes in wombat abundance were minimally influenced by emigration and immigration, which was supported by a consistent absence of wombats to the east of our study area, despite suitable habitat.

The burrow switching model, when fitted to the observed weekly flap-triggering data (Figure 4.1C), estimated the daily probability of burrow switching to be p = 0.128, (95% credible interval [0.106,0.154]). Thus, wombats were estimated to be switching burrows about once a

week (consistent with the literature, switching every 4 days on average; Skerratt *et al.* 2004a, Evans 2008). During the treatment phase of the study inactive burrows were visited each day by wombats with probability 0.062, (95% credible interval [0.055,0.070]). Thus, inactive burrows became active approximately once every 16 days.

Given this estimate of p and parameters provided by the literature (Table 4.1), we then simulated wombat-mite dynamics and identified sets of values for the unknown parameters that resulted in predictions consistent with the field observations (Figure 4.2). Consistency could be achieved for mean mite residence times, f, ranging from 10-20 days. When f = 10 days, consistency could only be achieved if delivery success during flap triggering, z_S, was low and in the range [0.25,0.33]. In this case, z_S, p_B and p_H were positively correlated, meaning our model could not easily distinguish between high delivery success and high rates of mite transfer, and vice-versa. Alternatively, when mite persistence was relatively high, f = 20 days, consistency could only be achieved when dose success was relatively high (e.g., z_S = 0.333) and rates of mite transfer were low (Table 4.2). Given these findings we identified three parameter scenarios that span the range of parameter values that produce realistic host-disease dynamics (Table 4.2). Importantly, for each of these parameter scenarios the host-disease model predicted that disease prevalence was low (2-3%) at the conclusion of the treatment regime (week 11), which is consistent with our field observations. Assuming that the model results accurately reflect disease dynamics in NNP, this suggests that our efforts fell just short of eradicating *S. scabiei* from the wombat population and surrounding environment. All of the simulations indicated that wombat numbers were stabilised during the course of the treatment; however, once treatment ended, signs of disease quickly re-emerged and spread through the population causing extinction of the study population (Figure 4.2).

Next, we evaluated the success of future realistic management solutions aimed at reducing prevalence of *S. scabiei* among wombat burrows at the end of the treatment period (week 11; Figure 4.3). As expected, mange prevalence was reduced by improving the probability that the treatment is successfully delivered to the host during flap triggering (Figure 4.3A), increasing the time that the treatment confers resistance to mange (Figure 4.3B), increasing the duration of the treatment program (Figure 4.3C), and increasing the proportion of burrows associated with treatment flaps (Figure 4.3D). The most impactful changes in our management strategy (inferred by steepness of slopes, Figure 4.3) is increasing the success of treatment delivery,

followed by extending the treatment program (Figure 4.3C). Surprisingly, the change with the weakest impact was increasing the duration of host resistance (Figure 4.3B), although this effect may be an underestimate of what would be expected in practice (see Discussion). These management conclusions appear robust because they were observed for all three parameter scenarios.

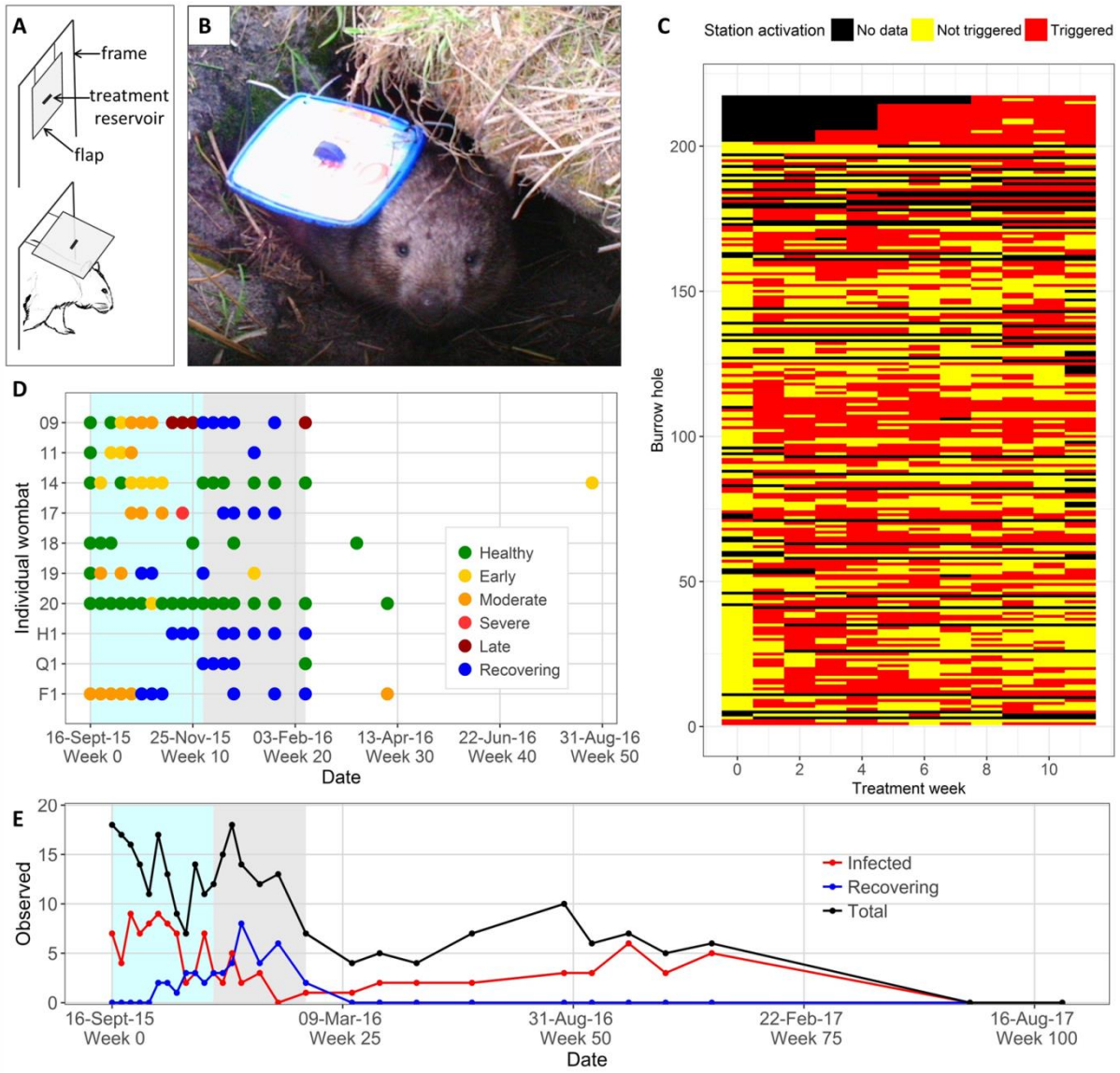

Figure 4.1. Empirical data from population-scale mange treatment in the field. Burrow flap treatment station (A and B) activation varied across weeks and burrows (C). The disease states of a subset (n=10) of wombats across survey weeks revealed that individual wombats exhibited signs of recovery through week 21 (light grey background shading, D and E). Raw data of wombat abundance during (light blue background shading) and following the treatment experiment showed short-term treatment success.

Table 4.2. Three scenarios defined by the set of model parameter values that have high uncertainty. Each scenario results in the host-disease model predicting dynamics consistent with the field observations.

Parameter	Symbol	Scenario		
		A	B	C
Mean mite survival in inactive burrow (days)	f	10	10	20
Prob. an infected burrow infects a wombat	q_B	0.5	0.333	0.2
Prob. a highly infected wombat infects a burrow	q_H	0.6	0.5	0.25
Prob. a triggered flap successfully treats a wombat	z_S	0.333	0.25	0.333

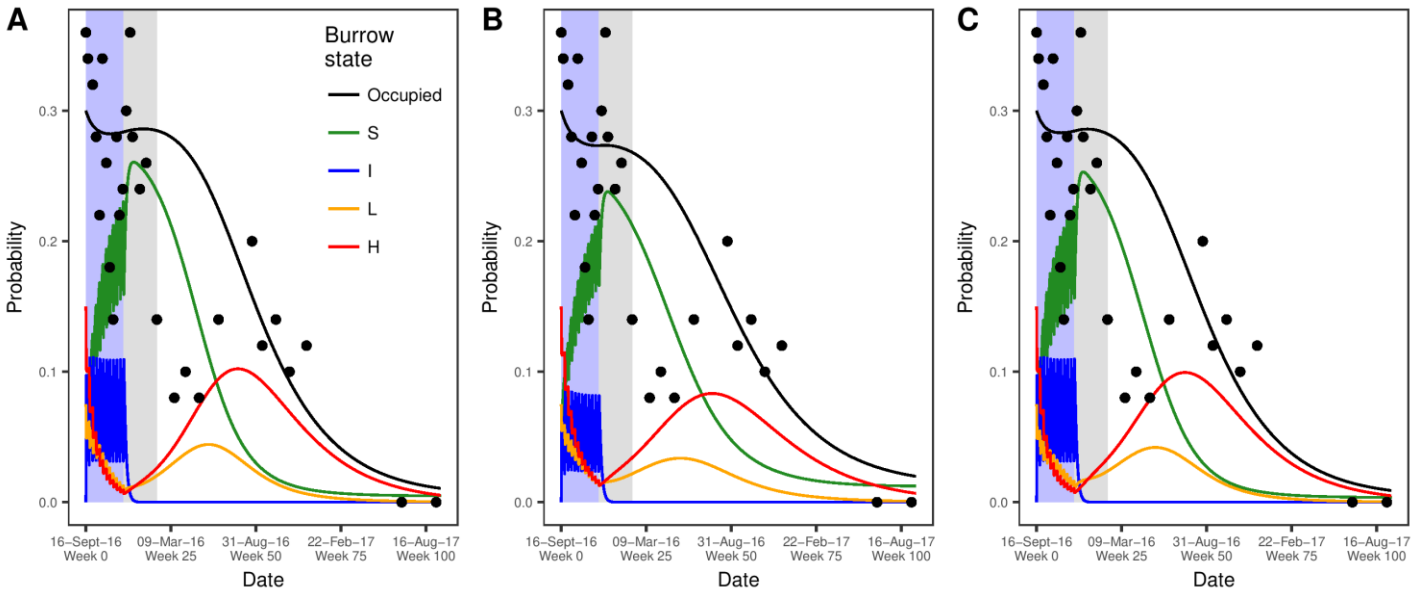

Figure 4.2. Simulated changes in wombat abundance over 2 years (lines). Black lines depict the proportion of burrows simulated to be occupied by wombats and colours identify the state of these wombats (S = susceptible to mange, I = treated and currently immune to mange, L = low mange infection, H = high mange infection). All simulations assumed the fixed parameters presented in Table 4.1. Panels show host-disease dynamics for the three parameter scenarios presented in Table 4.2. Circles depict changes in the relative number of wombats observed at NNP during the first survey night (c.f. Figure 4.1E). The light blue box encompasses the treatment weeks and the light grey box highlights weeks post-treatment where recovering wombats were observed.

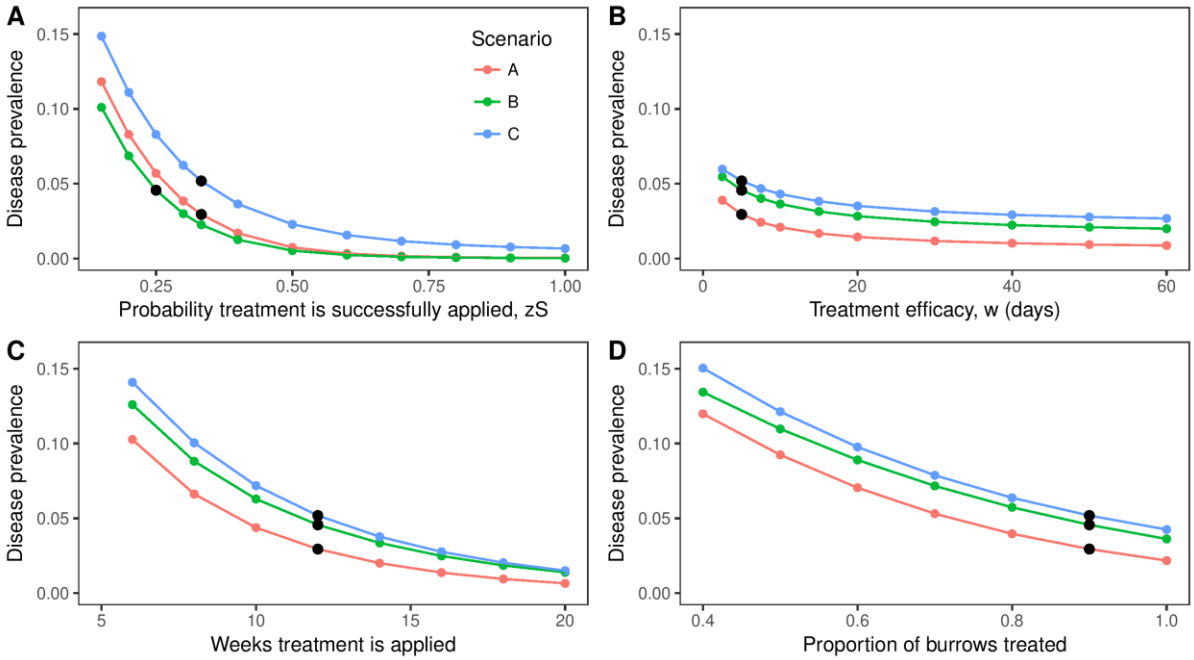

Figure 4.3. Predicted disease prevalence among burrows on the last day of treatment for the three parameter scenarios (coloured lines) defined in Table 4.2. The four panels depict possible alterations to management strategies/solutions, including (A) increasing the efficiency of treatment delivery (z_S), (B) increasing the duration that the treatment remains effective (confers treatment to) in the host (w), (C) extending the treatment duration (T), and (D) increasing the burrow coverage (v). Black circles correspond to dynamics depicted in Figure 4.2.

4.5 Discussion

Here, we have shown that a population-scale treatment to eradicate environmentally transmitted *S. scabiei* from bare-nosed wombats during an epizootic was almost successful, and by combining our field study with a system-specific host-disease model we were able to improve our understanding of how prevalence of this pathogen in the environment is linked to host movement behaviour, host-mite interactions, and management. Currently, most wildlife disease control attempts are justifiably based on knowledge from field and laboratory research (Joseph *et al.* 2013). How this knowledge is best translated to a field application, however, is not always clear. Our host-disease model identified treatment delivery success at burrows and treatment duration as the two most important determinants of the success of a treatment program for eradicating mange among wombats.

Host-disease models typically focus on describing changes in the disease status of the host, but given that mite transmission has an environmental component, disease dynamics in our system could be better understood by focusing on the disease status of burrows and considering hosts as the disease vector. This switch in focus is akin to treating disease as if it were a meta-population among burrows, and a somewhat similar approach has been applied to understanding drivers of plague dynamics among prairie dog colonies (Snäll *et al.* 2008). An interpretation of our approach is that mite persistence requires an infected burrow to, on average, replace itself with another infected burrow before the local mite population goes extinct. Thus, the calculation of R_0 for mange should be based on expected changes in disease status of the burrow, not just the host. Our host-disease model suggests that persistence of mange is largely dependent on the frequency of wombat burrow switching; if burrow switching is sufficiently low, then the disease cannot persist, as rates of contact between infected burrows and susceptible wombats declines below that needed to establish new burrow infections. Treatment of wombats using remote dosing stations can be effective because it prevents mites from being picked up from infected burrows and subsequently transmitted to uninfected burrows.

Our model predicts that when the treatment program concludes, mange prevalence should (in our epizootic case) eventually return to high levels and the wombat population will go extinct, unless mange has been eradicated from burrows. Given that mange does not always lead to

an outbreak, our model suggests that local long-term persistence of mange may be due to temporal or spatial variation in (i) the mean duration of mange persistence in unoccupied burrows, (ii) burrow switching rates, and (iii) the likelihood of mite transfer between burrows and their wombat occupants. Understanding these aspects of wombat biology and how they vary within and among populations is therefore necessary for understanding the epidemiology of this host-parasite system.

In practice, we expect burrow coverage to be one of the most difficult parameters to manipulate due to the difficulty in identifying all active burrows in the landscape. Although not presented, concurrent improvements in treatment parameters (e.g., program duration and duration of host protection against mites) have an additive effect in reducing mange prevalence. Concurrent improvements may be necessary for management success for systems where burrow coverage is low. Among the three model scenarios that were consistent with our observational data (Figure 4.2), we predict successful management (reducing mange prevalence) to be most challenging to achieve when mites persist for long periods in the burrow in the absence of a host (Figure 4.2, Scenario 3).

Remote administration methods are powerful tools for disease control. For example, the remote method of oral baiting has been used to vaccinate for rabies in European foxes (Cliquet and Aubert 2004, Freuling *et al.* 2013), plague in North American prairie dogs (Salkeld 2017), and bovine tuberculosis in brushtail possums (Tompkins *et al.* 2009), and has been largely successful. In contrast, direct administration methods, such as capture-inject-release, are more invasive and their success relies on the ability to trap animals, but delivery efficacy is high. While the development of remote treatment administration methods (i.e., burrow flaps) has been a major revolution in control efforts for *S. scabiei* in wombats – creating protected/ resistant hosts as the environmental reservoir dies out – here we show that delivery success of treatment is the greatest limitation to population-scale pathogen eradication. Our modelling revealed surprisingly low success of treatment delivery (less than 50%, and likely nearer to 33%), which is difficult to observe when using remote methods. We suspect this is primarily due to the delivery method (burrow flap), which administers a single dose indiscriminately and anecdotally can be avoided or disrupted by wombats. It is also difficult to quantify the treatment dose that is delivered, but a dose of less than 2 mL may be insufficient to clear infection or confer complete protection to the host (Death *et al.* 2011). Improvements in

delivery success appear to be the single most important factor in control of *S. scabiei*. Thus, our research supports advances in delivery methods that allow for targeted administration or administration of multiple doses to increase treatment efficacy. More broadly, technological advancements in remote delivery of treatment and vaccines to wildlife may be the most impactful tool for enhancing disease control across systems.

There is currently no proven method to directly eliminate *S. scabiei* from environments supporting infection to wildlife hosts (such as bedding chambers in wombat burrows in our system). Fumigation of wombat burrows has been proposed, but poses challenges both logistical (e.g., demonstrating mite killing in difficult to access burrow environments) and ethical (e.g., exposure of wombats in burrows to fumigation), that make direct environmental control presently unrealistic. In contrast, enhancements in treatment methodology (delivery success) or treatment formulations (the duration of protection conferred) are more immediately feasible. Our model suggests that a longer lasting treatment would only have a moderate effect in reducing mange prevalence, and it is important to acknowledge that in practice, longer lasting treatments would likely also allow for greater effort to be invested in treatment coverage of the population. Recent advances in longer lasting treatments for control of *S. scabiei* and other ecto-parasites (e.g., fluralaner, a newly developed, long-lasting, ecto-parasiticide) may be candidates for wombats (Taenzler *et al.* 2016). Thus, an improved pharmaceutical agent in conjunction with other management changes (i.e., increased delivery success) should result in a more effective overall treatment program.

One potential caveat of this research is the possibility that healthy wombats were underrepresented during dusk surveys. In Tasmania, healthy bare-nosed wombats exhibit both diurnal and nocturnal activity, with a trend for increased observations of healthy wombats at night, though this varies with location (DPIPWE 2017a). While our pre-dusk survey efforts may slightly bias diseased individuals (Hartley and English 2005, Simpson *et al.* 2016), our observations broadly capture the population and disease trends. We expected that wombat behaviour would become increasingly nocturnal as individuals recovered from disease following treatment, which likely explains the decrease in number of wombats observed after the treatment regime ended, as recovering individuals peaked. Accordingly, wombat observations increased as mange disease became more prevalent after the effect of treatment was no longer obvious (no remaining recovering individuals).

This study highlights logistical challenges in the control and eradication of pathogens that utilize environmental reservoirs in transmission. While this study has focussed on mange in wombats, it's approach and findings apply more broadly. For example, sarcoptic mange control in red foxes may require efforts to eradicate the mites from both the host and potential environmental reservoirs (den), similar to our disease management in bare-nosed wombats. In contrast, control efforts in host systems without a plausible environmental transmission route, such as the gregarious Spanish ibex, may be best directed towards establishing widespread host resistance. Additional complexities can also occur when other hosts contribute to transmission (e.g., canids acting as reservoirs which spread mange to other marsupials on the Australian mainland), and research examining how multi-host dynamics can be accommodated into *S. scabiei* disease control programs would be valuable. Our research fills knowledge gaps in wildlife disease management, specifically the importance of effective treatment delivery and drug viability, and bridges disease theory with practice. Furthermore, our results may have broad implications for other wildlife diseases with environmental transmission, including conservation critical diseases such as white nose syndrome and chytridiomycosis.

Environmental disease reservoirs continue to be recognized as key factors in disease outbreaks, pathogen persistence, and on-going transmission in humans (King *et al.* 2008, Zhao *et al.* 2012), domestic animals (Georgsson *et al.* 2006, Allerson *et al.* 2013), and wildlife systems (Breban *et al.* 2009, Almberg *et al.* 2011, Park 2012, Wang *et al.* 2012), and is one of the few mechanisms that allows for disease-induced host extinction events (De Castro and Bolker 2005). It is imperative to understand and employ effective control of environmentally persistent pathogens. Using the infection status of an environmental reservoir in combination with host reservoir switching behaviour to model disease transmission and prevalence is novel, and to our knowledge has not been utilised before. Our model can incorporate these concepts to inform management effort required to control disease prevalence in a given system. This methodology may be applied to other disease systems where the pathogen is capable of surviving in the environment and the host may engage in reservoir switching behaviour, facilitating transmission. One such system may include white-nosed syndrome (causative agent *Pseudogymnoascus destructans*) in North American hibernating bat species, whereby the fungal pathogen can survive in suitable conditions for ≥5 years (Hoyt *et al.* 2015) and host

switching among hibernacula between (and within) years impacts the disease status of the environmental reservoirs (hibernacula). This system, like our own, may involve multiple host species. Our model can be readily extended to incorporate multiple hosts and provides a framework for assessing alternative management strategies for regulating diseases that have an environmental component of transmission.

I. Field site

Narawntapu National Park is located on the north-central coast of Tasmania (Figure S4.1A). The park is bordered by Bass Strait to the north, Port Sorell to the west and south, and Briggs Regional Reserve to the east. Wombat burrows were surveyed in a 1.1 km management area in the west of the park (burrows in grey; Figure S4.1B), and those deemed active, based on the evidence of wombat presence, were equipped with a burrow flap (red; Figure S4.1B). Many of the unused burrows were located along a drainage ditch line in the south, separating the park from Port Sorell. Active burrows were located in large, grass paddocks (south of grey line) and in the surrounding vegetation (eucalyptus species, shrubs, and native grass species; outside of grey line).

Figure S4.1: (A) Location of the study site, and (B) location of the burrows identified during the study (circles); active burrows associated with a flap (red), inactive burrows (grey).

II. Wombat trapping, anaesthesia, and processing to equip wireless identification devices (ear tags)

Individuals were trapped on foot using large, mesh nets and were anaesthetized (zolazepam /tiletamine, Zoletil, Virbac, dose: 3-4mg kg^{-1} and medetomidine, dose: 40 µg kg^{-1} intramuscular [IM] injection; Ethics Approval Permits A14670, FA15122) for processing, whereby ear biopsies were taken and WIDs were fitted. Post-processing, wombats were administered a sedative reversal (atipamezole, dose: 40 µg kg^{-1} IM) and held in wire Mascot animal traps, padded and insulated with hessian sacks, for 6-12 hours until fully recovered from anaesthesia. Wombats were then released at the site of capture.

III. Burrow switching model

This section provides details on how the daily probability a wombat switches its burrow was estimated from the weekly checks of treatment flaps. First, the flap data is described. Next, a stochastic model is developed that asks the question: what is the probability of observing the flap data if wombats switch burrows daily with probability p. Parameter estimates and their uncertainty, derived from a Bayesian fitting approach, are then presented.

III.I Flap data

Consider a wombat burrow where a flap is placed over the entrance of the burrow and the flap is associated with a treatment applicator. When an animal enters a burrow the flap is triggered and the applicator dispenses a dose of treatment. Applicators only dispense a single dose before needing to be refilled. Burrow treatment involves filling the applicator with the treatment solution and returning D days later to see whether or not the lid has been moved. If the lid is moved, then it indicates that at least once during the previous D days the flap was triggered, most likely by a wombat either entering or exiting the burrow. Suppose the burrow is monitored for J contiguous time periods, each of length D days, and at the start of each period a new treatment is applied (i.e., the flap is set). Let $y_j = \{T, \bar{T}\}$ denote if the flap was triggered or not during time period j. The data associated with the burrow is the vector $\vec{y} = \{y_1, y_2, \ldots, y_J\}$. The complete data set consists of a vector of triggering observations for each burrow monitored.

III.II Statistical model of burrow switching and parameter estimation

Burrow usage is modelled by assuming that wombats switch burrows each day with probability p and unoccupied burrows are visited each day with probability v. The probability of observing a flap triggering event during a time period depends on whether or not the burrow was occupied the night before the start of the time period. This uncertainty in occupancy is a hidden state variable, which makes estimating p and v more difficult.

Suppose at the start of monitoring, unknown to the observer, occupancy the night prior is $x = \{0,1\}$, indicating not occupied and occupied, respectively. Let d denote the number of days after the lid was last set ($1 \leq d \leq D$). Each day the burrow may be in one of three states: (1) flap has not been triggered, (2) flap has been triggered but the burrow was not occupied the night prior, or (3) the flap has been triggered and the burrow was occupied the night prior. Let $q_{j,x}^{(d)}$ denote the probability that the burrow is in state j, d days after the flap was last set, and occupancy on the night prior to day $d = 1$ is x. After the first day these probabilities are related by:

$$q_{1,x}^{(d+1)} = (1-v)q_{1,x}^{(d)},$$

$$q_{2,x}^{(d+1)} = (1-v)q_{2,x}^{(d)} + p(1-v)q_{3,x}^{(d)},$$

$$q_{3,x}^{(d+1)} = v(q_{1,x}^{(d)} + q_{2,x}^{(d)}) + (1-p+pv)q_{3,x}^{(d)},$$

for $1 \leq d \leq D-1$. However, for the first day after last setting the lid the probabilities depend on x (i.e., prior occupancy before the lid was last set), according to:

$$q_{1,0}^{(1)} = 1-v,$$

$$q_{2,0}^{(1)} = 0,$$

$$q_{3,0}^{(1)} = v,$$

$$q_{1,1}^{(1)} = p(1-v),$$

$$q_{2,1}^{(1)} = 0,$$

$$q_{3,1}^{(1)} = 1-p+pv.$$

Let $\Pr(\vec{y}, x'|x)$ denote the probability of observing the flap data $\vec{y}$, given that initially the burrow had occupancy, x, and immediately after the data were recorded occupancy was x'. After the first period of data collection ($i = 1$):

$$\Pr(T, 1|x) = q_{3,x}^{(D)},$$

$$\Pr(T, 0|x) = q_{2,x}^{(D)},$$

$$\Pr(\bar{T}, 0|x) = q_{1,x}^{(D)}.$$

For future time periods these conditional probabilities are related according to:

$$\Pr(\vec{y} \cup T, 1|x) = \Pr(\vec{y}, 1|x)q_{3,1}^{(D)} + \Pr(\vec{y}, 0|x)q_{3,0}^{(D)}$$

$$\Pr(\vec{y} \cup T, 0|x) = \Pr(\vec{y}, 1|x)q_{2,1}^{(D)} + \Pr(\vec{y}, 0|x)q_{2,0}^{(D)}$$

$$\Pr(\vec{y} \cup \bar{T}, 0|x) = \Pr(\vec{y}, 1|x)q_{1,1}^{(D)} + \Pr(\vec{y}, 0|x)q_{1,0}^{(D)}$$

Note that $\Pr(\vec{y} \cup \bar{T}, 1|x) = 0$ for data collected across one or more weeks, because the burrow must be unoccupied at the end of sampling if the flap was not triggered during the last observation period.

Similarly, let $\Pr(\vec{y}|x)$ denote the probability of observing the flap data $\vec{y}$, given that immediately before the data were collected the burrow had occupancy, x. These two probabilities (i.e. $x = 0,1$) can be calculated using:

$$\Pr(\vec{y}|x) = \Pr(\vec{y}, 0|x) + \Pr(\vec{y}, 1|x).$$

If the probability the burrow was initially occupied is f, then the probability of observing the data $\vec{y}$ is

$$\Pr(\vec{y}) = (1 - f)\Pr(\vec{y}|0) + f\Pr(\vec{y}|1).$$

Assuming all burrows are intrinsically identical, our movement model is defined by three unknowns, $\vec{\theta} = \{f, v, p\}$. Let $\vec{y}_i$ denote the flap data associated with burrow i ($1 \leq i \leq I$). The likelihood of the burrow switching model, given all the burrow data, is

$$L(\vec{\theta}|\text{data}) = \prod_{i=i}^{I} \Pr(\vec{y}_i).$$

Given this likelihood, we then used Bayesian Monte-Carlo methods to estimate the posterior distributions for the three model parameters. Specifically, we coded the likelihood function using the R package, *rstan*. For all three parameters we set uniform priors with plausible ranges

that had very little influence on the posterior parameter estimates. We considered 5 chains, each run for 1000 iterations, and we disregarded the first 500 iterations, by which time it was clear that parameter estimates were consistent with their posterior distributions.

Having estimates for p and v, we can estimate the proportion of burrows in use during the survey that were occupied on any one night, using:

$$\frac{n}{N} \approx \frac{v}{p+v(1-p)}.$$

To check for consistency, we simulated wombat movement according to our model and compared the flap triggering data it produced with the observed flap data. The simulation model incorporated the most-likely model parameter estimates and was coded using the R programming language (R Core Team, 2018).

III.III Results

Observed burrow triggering frequencies for 209 burrows over a 12 week period are presented in Figure S4.2. Week 2 data were not included in the analysis as flap triggering was low and inconsistent with the remaining weeks of observations; we suspect that wombat disturbance and lack of experience setting up the flaps led to this initial discrepancy. Also, triggering of burrow holes 202–209 were very high across the weeks monitored and inconsistent with the other burrows (Figure S4.2). These burrows became available to wombats later in the study as water levels dropped. As patterns of occupancy for these relatively few burrows were inconsistent with the majority of burrows, they were also excluded from the fit.

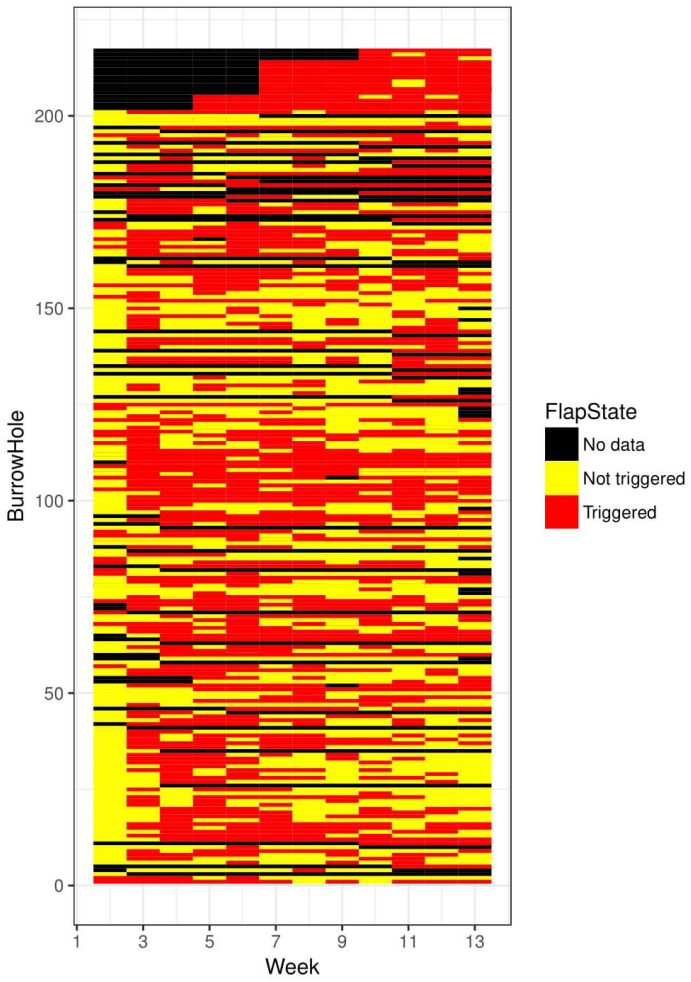

Figure S4.2: Flap triggering data for 209 burrow holes surveyed over a 12 week period (weeks 2–13).

A summary of the sample sizes and weekly triggering rates for the data included in the fit are presented in Figure S4.3. Each week approximately 180 burrows were monitored and just over a half of these were triggered each week, although this fraction showed a slight but steady decline during the course of the study (Figure S4.3).

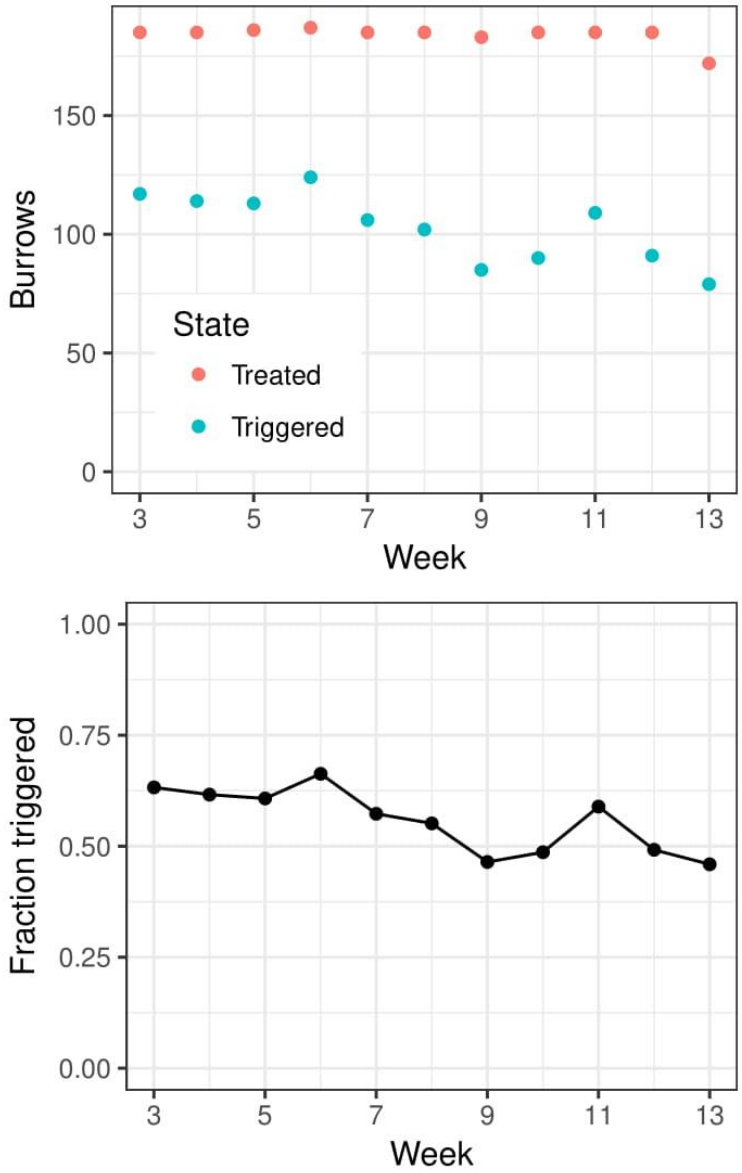

Figure S4.3: Summary of the weekly number of treated burrows and the number of those that were triggered (top) and the corresponding triggering rates (bottom). Only the first 201 burrows presented in Figure S4.2 were considered, and data from week 2 were discarded as there appeared to be lower triggering weeks.

Parameter estimates and their uncertainty are presented in Table S4.1. The fitted model suggested that each day wombats switched burrows with probability $p \approx 0.128$. Each day, unoccupied burrows were visited by wombats with probability $v \approx 0.062$. The proportion of burrow holes that were estimated to be occupied the night before flaps were first applied to the hole was $f \approx 0.573$ (i.e., over half). Given these parameters, the model estimated that the fraction of burrow holes in use each day (n/N) was 0.353, indicating that the initial placement of flaps was not random but biased towards occupied holes (as expected). Importantly, these parameter estimates were able to produce simulated burrow usage that was consistent with the observed data (c.f., Figures S4.2, S4.4).

Table S4.1: Estimated model parameters. Median and 95% credible intervals for the three estimated model parameters are presented. Also shown is the estimated fraction of burrows occupied by wombats each night, n/N.

Parameter	2.5%	50%	97.5%
p	0.106	0.128	0.154
v	0.055	0.062	0.070
f	0.442	0.573	0.686
n/N	0.311	0.342	0.374

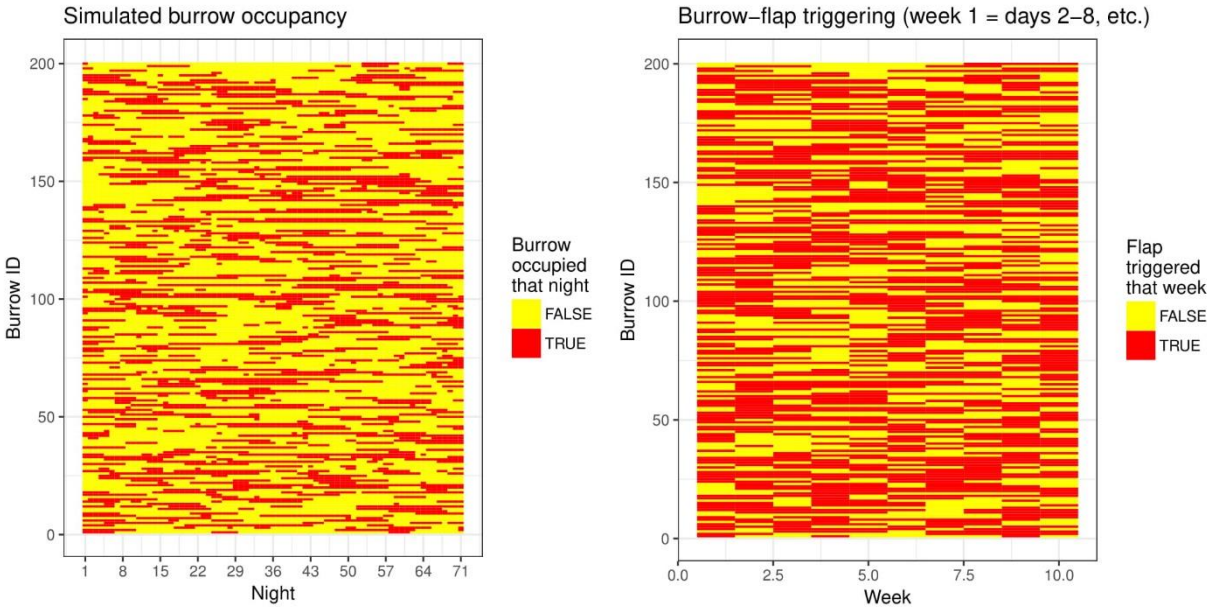

Figure S4.4: (Left) Simulated daily burrow usage for 200 burrows over a 10-week period when 70 wombats (35% burrow occupancy) switch burrows each day with probability $p = 0.128$. (Right) Simulated flap triggering observations if flap monitoring and resetting is performed weekly.

IV. Host-disease model

This section describes in detail the equations used to simulate mite persistence among a local population of wombats subject to the treatment program described in the main text.

IV.I Model framework

One of the most common ways to model host-disease dynamics is to assign a set of disease states to the hosts and then track how the number of hosts in each state changes over time. This general approach is often referred to as a susceptible-infected-recovered (SIR) model. If natural recovery from disease is not possible (or unlikely), then the R-state can be removed, which is in fact the case for our wombat-mange system. This approach can be easily modified to include a treated state that implies temporary host immunity to disease.

Typically, host-disease dynamics are modelled using a set of coupled ordinary differential equations (ODEs), which are often simple to write down. One drawback of this approach, however, is that the parameters describe continuous rates, which are not always straightforward to estimate directly from data (e.g., the rate that hosts and the disease come into contact). In the previous section we were able to quantify wombat movement behaviour in terms of a daily probability of burrow switching. Ideally, this parameter should be integrated directly into the host-disease model. Also, the ODE approach is particularly problematic when modelling our system because treatment is not applied continuously in time. There are two important time-scales operating in our system: wombats are potentially switching burrows daily but burrows are only treated weekly.

To overcome these time-scale and parameterisation issues inherit to our system we adopted a time-step approach in favour of the continuous-time ODE approach, where the time-step is a day. A necessary consequence is that the set of equations, needed to describe the important processes operating in our system, is much more complex than is typically encountered with ODEs. Although the model may appear complex it is just the result of a book-keeping exercise that tracks probabilities associated with all possible outcomes resulting from a set of simple biological processes. Details of the model are presented below.

IV.II System state

We consider an environment composed of many burrows. Each day, burrows are in a state that is defined in terms of three variables: (1) if it is occupied by a wombat, (2) if it is occupied by mites, and (3) if it covered by an un-triggered flap containing effective treatment. Each night a burrow may be occupied by, at most, a single wombat, and wombats may be in one of four states: susceptible to mite infection (S), currently treated and protected from mite infection (I), experiencing low mite infection (L), or experiencing high might infection (H). Only highly infected hosts are visibly infected in the field. Infected wombats may transfer mites to burrows and infected burrows may transfer mites to wombats. Treated flaps are always triggered when a wombat moves in or out of the burrow, however a triggering event does not always result in the wombat being treated. Each day there is a probability that the treatment in an un-triggered flap becomes ineffective.

The probability a randomly chosen burrow on day t has wombat occupancy status i = {U, S, I, L, H}, disease occupancy status j = { D, $\overline{\mathrm{D}}$ }, and treatment status k = { T, $\overline{\mathrm{T}}$ }, is denoted $P_{i,j,k,t}$. Here, U indicates that the burrow is unoccupied, and the bar notation indicates the absence of either disease or effective treatment. There are 20 possible burrow states.

Wombat population density (per burrow) on day t is given by

$$N_t = \sum_{i \neq \mathrm{U},j,k} P_{i,j,k,t}.$$

Similarly, disease density is given by

$$D_t = \sum_{i,j=\mathrm{D},k} P_{i,j,k,t}.$$

IV.III State dynamics

Each day a sequence of events occurs, which may change the state of the burrows. Here, we assume the following sequence:

1. Wombats die.
2. Unoccupied, infected burrows lose disease.
3. Infected wombats move from low to high.
4. Treated wombats lose their immune status.
5. Treated burrows lose treatment viability.
6. Burrows gain effective treatment.
7. Some wombats move and change burrows.
8. Wombat offspring gain independence.

Let $P^{(i)}_{i,j,k,t}$ denote the distribution of burrow states on day t after event i. This distribution at the start of the following day is calculated using $P_{i,j,k,t+1} = P^{(8)}_{i,j,k,t}$. Host-disease dynamics are robust to the ordering of the events.

IV.III.I Wombat deaths

Healthy wombats (states: S and I) live, on average, L days (i.e., they die each day with probability $m_0 = 1/L$). Wombat life-expectancy is about 10 years (i.e. $L = 10 \times 365$ days). Wombats in the low and high disease state live, on average, for a further L_{L} and L_{H} days, respectively. In these states the daily probability of death are $m_i = 1/L_i$. Wombats in the high disease state are expected to survive, on average, a further $L_{\mathrm{L}} = 90$ days. Here, we assume that life-expectancy is not affected when in the low disease state (i.e. $L_{\mathrm{L}} = L$). After deaths we have:

$$P^{(1)}_{\mathrm{U},j,k,t} = P_{\mathrm{U},j,k,t} + m_0 P_{\mathrm{S},j,k,t} + m_0 P_{\mathrm{I},j,k,t} + m_{\mathrm{L}} P_{\mathrm{L},j,k,t} + m_{\mathrm{H}} P_{\mathrm{H},j,k,t}$$

$$P^{(1)}_{\mathrm{S},j,k,t} = (1 - m_0) P_{\mathrm{S},j,k,t}$$

$$P^{(1)}_{\mathrm{I},j,k,t} = (1 - m_0) P_{\mathrm{I},j,k,t}$$

$$P^{(1)}_{\mathrm{L},j,k,t} = (1 - m_{\mathrm{L}}) P_{\mathrm{L},j,k,t}$$

$$P^{(1)}_{\mathrm{H},j,k,t} = (1 - m_{\mathrm{H}}) P_{\mathrm{H},j,k,t}$$

These transition probabilities are depicted in Figure S4.5. Note that wombat deaths do not change the treatment status or disease status of the burrow.

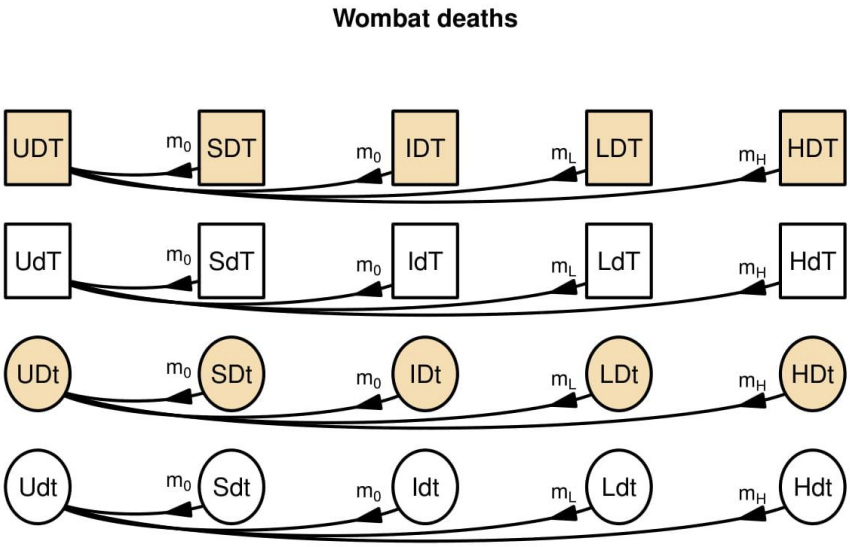

Figure S4.5: Daily probabilities of moving between burrow-states (circles and squares) due to wombat deaths. Refer to Section IV.II for descriptions of the state descriptors: U, D, d, T, t, and S. States where the burrow has an untriggered and viable flap (T), and those without (t), are depicted by squares and circles, respectively. Shaded states indicate that the burrow is occupied by a mite population (D). The first column depicts states where the burrow is unoccupied by a wombat (U) and the four remaining columns separate the state of the wombat that occupies the burrow: S, I, L, H.

IV.III.II Disease persistence in burrows

On average, burrows that are not occupied by diseased animals take f days to become disease free (i.e., each day the probability a burrow becomes disease-free is $p_{\overline{\mathrm{D}}} = 1/f$). As only diseased burrows that are either unoccupied, or occupied by either susceptible or immune wombats, can lose disease, we have:

$$P^{(2)}_{i,\mathrm{D},k,t} = (1 - p_{\overline{\mathrm{D}}})P^{(1)}_{i,\mathrm{D},k,t}$$

$$P^{(2)}_{i,\overline{\mathrm{D}},k,t} = P^{(1)}_{i,\overline{\mathrm{D}},k,t} + p_{\overline{\mathrm{D}}}P^{(1)}_{i,\mathrm{D},k,t}$$

for $i \in$ {U, S, I}. The other states remain unchanged during this event (i.e. $P^{(2)}_{i,j,k,t} = P^{(1)}_{i,j,k,t}$) (see Figure S4.6).

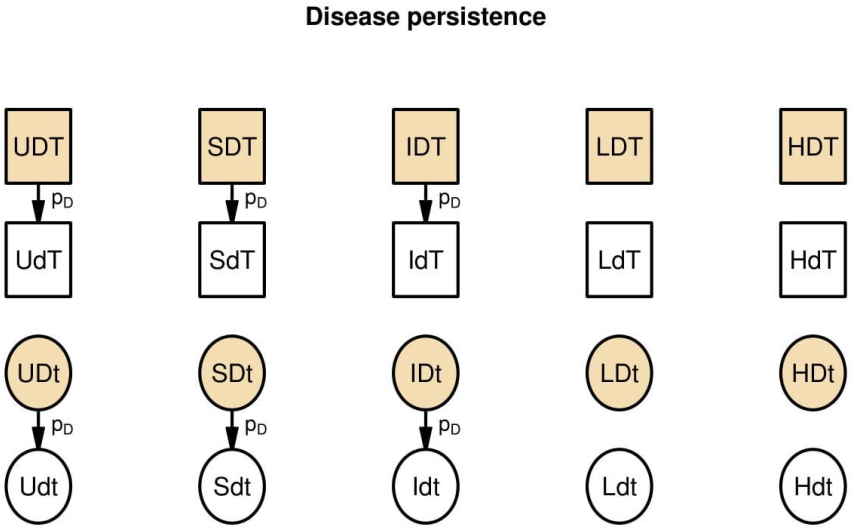

Figure S4.6: Daily probabilities of moving between states due to local mite extinctions within burrows (p_D).

IV.III.III Wombat disease progression

On average, it takes d days for wombats in the low-disease state to move to the high-disease state (i.e., each day the probability a low-diseased wombat moves to the high-disease state is $p_{\mathrm{H}} = 1/d$). Burrows occupied by diseased wombats are updated using:

$$P^{(3)}_{\mathrm{L},j,k,t} = (1 - p_{\mathrm{H}})P^{(2)}_{\mathrm{L},j,k,t}$$

$$P^{(3)}_{\mathrm{H},j,k,t} = P^{(2)}_{\mathrm{H},j,k,t} + p_{\mathrm{H}}P^{(2)}_{\mathrm{L},j,k,t}$$

The other states remain unchanged (Figure S4.7).

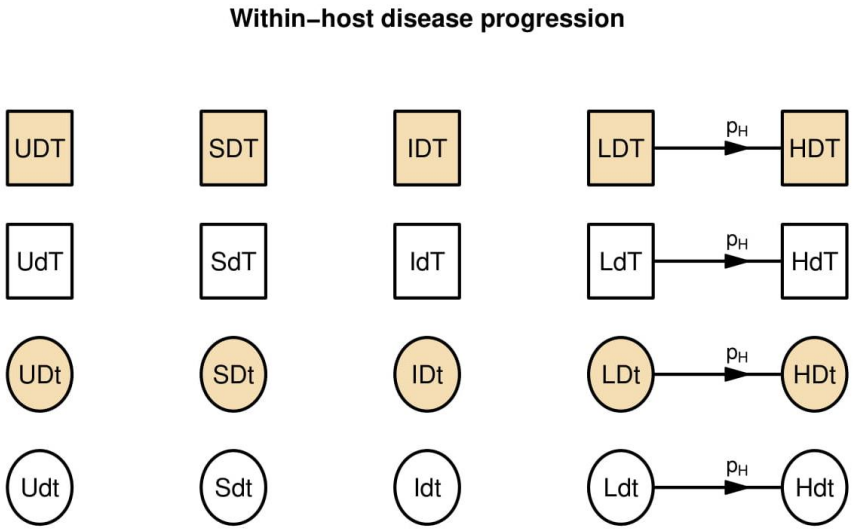

Figure S4.7: Daily probabilities of moving between states due to mite infection increasing on wombat hosts (p_H).

IV.III.IV Treatment loss for wombats

On average, treated wombats keep their protection from disease for w days (i.e., they lose immunity each day with probability $P_{\mathrm{S}} = 1/w$), which implies:

$$P^{(4)}_{\mathrm{I},j,k,t} = (1 - p_{\mathrm{S}})P^{(3)}_{\mathrm{I},j,k,t}$$

$$P^{(4)}_{\mathrm{S},j,k,t} = P^{(3)}_{\mathrm{S},j,k,t} + p_{\mathrm{S}}P^{(3)}_{\mathrm{I},j,k,t}$$

The other states remain unchanged (Figure S4.8).

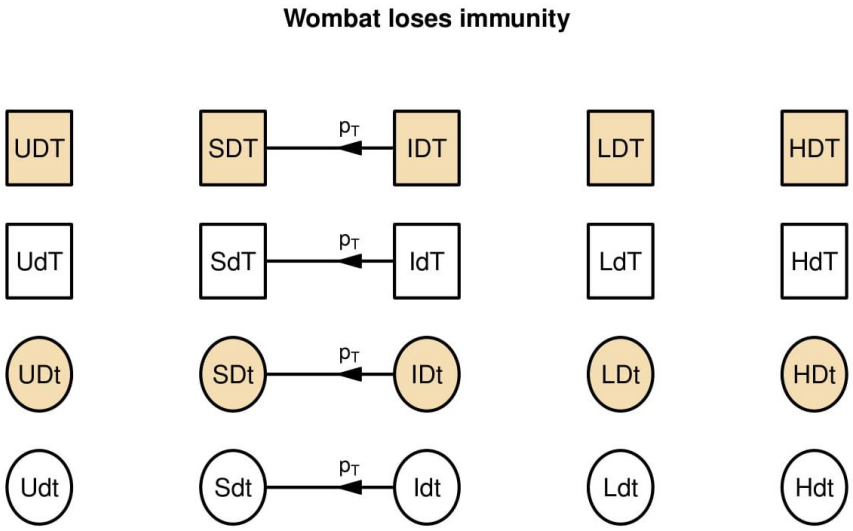

Figure S4.8: Daily probabilities of moving between states due to wombat hosts losing short-term mite immunity provided by the treatment (p_T).

IV.III.V Treatment loss for burrows

The treatment placed on un-triggered flaps loses viability each day with probability $p_{\overline{\mathrm{T}}}$. In this case, all states are affected by the event (Figure S4.9):

$$P^{(5)}_{i,j,\mathrm{T},t} = (1 - p_{\overline{\mathrm{T}}})P^{(4)}_{i,j,\mathrm{T},t}$$

$$P^{(5)}_{i,j,\overline{\mathrm{T}},t} = P^{(4)}_{i,j,\overline{\mathrm{T}},t} + p_{\overline{\mathrm{T}}}P^{(4)}_{i,j,\mathrm{T},t}$$

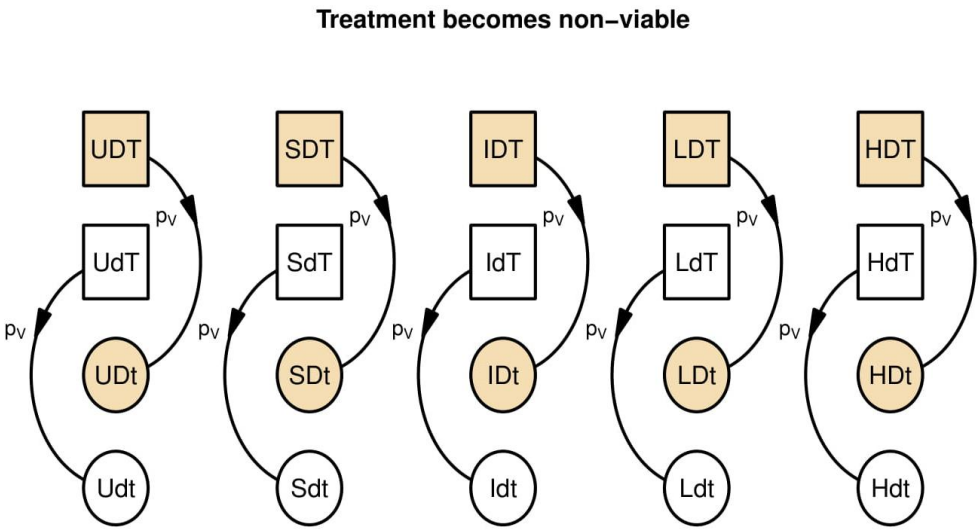

Figure S4.9: Daily probabilities of moving between states due to the treatment on the non-triggered flaps losing viability (p_V).

IV.III.VI Treatment gain

On day t a random proportion, v_t, of burrows are treated, which leads to:

$$P^{(6)}_{i,j,\overline{\mathrm{T}},t} = (1 - v_t)P^{(5)}_{i,j,\overline{\mathrm{T}},t}$$

$$P^{(6)}_{i,j,\mathrm{T},t} = P^{(5)}_{i,j,\mathrm{T},t} + v_t P^{(5)}_{i,j,\overline{\mathrm{T}},t}$$

(see Figure S4.10). Here, new treatment is applied weekly, so $v_t = 0$ on most days.

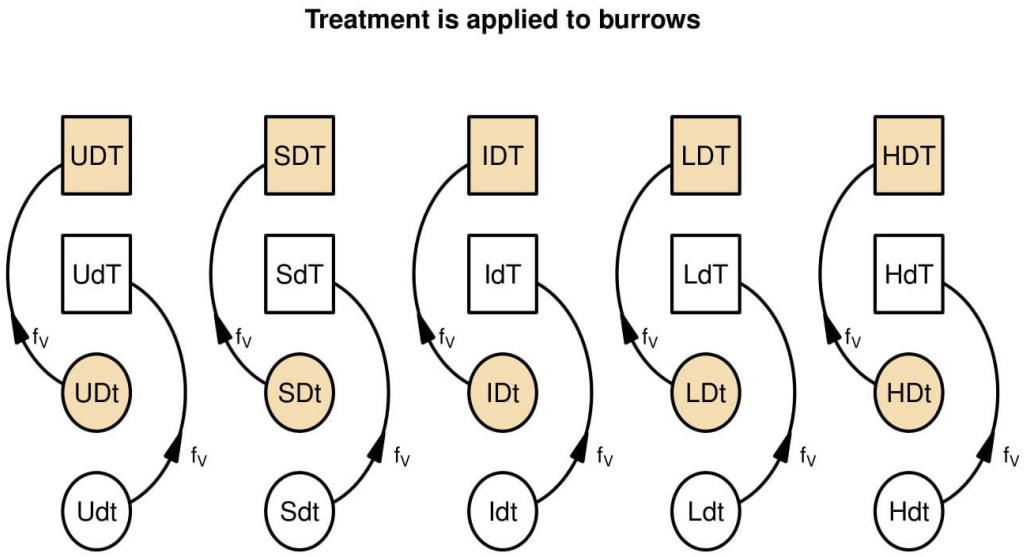

Figure S4.10: Daily probabilities of moving between states due to flaps being checked and fresh treatment being applied to burrow flaps (f_V).

IV.III.VII Wombat burrow switching

Each day wombats leave their burrow to forage. After a foraging bout, wombats may choose to return to the same burrow that they occupied the night before or they may choose to switch burrows. Suppose that the probability a wombat switches burrows is p and new burrows are chosen at random (i.e., they do not know, or care, if a burrow contains disease or treatment). In either case, if a diseased wombat in state $i \in \{ \mathrm{L}, \mathrm{H} \}$ enters a disease-free burrow it transfers disease to the burrow with probability q_i. Conversely, if a susceptible wombat enters a diseased burrow the wombat contracts the disease and enters the low disease state with probability q_{B}. When a wombat enters a treated burrow it always triggers the flap and the probability the treatment is successfully transferred to the wombat (i.e., moves the wombat to the temporary immune state, I), when the wombat is in state $i \in \{\mathrm{S}, \mathrm{L}, \mathrm{H}\}$, is denoted z_i.

The distribution of burrow states after the wombats that have decided to not switch burrows and have returned to their burrow, but switchers are still searching for a new burrow, is given by:

$$P'_{i\neq \mathrm{U},j,\mathrm{T},t} = 0$$

$$P'_{\mathrm{U},j,\mathrm{T},t} = P^{(6)}_{\mathrm{U},j,\mathrm{T},t}$$

$$P'_{\mathrm{U},j,\overline{\mathrm{T}},t} = P^{(6)}_{\mathrm{U},j,\overline{\mathrm{T}},t} + p\sum_{i\neq \mathrm{U},k} P^{(6)}_{i,j,k,t}$$

$$P'_{\mathrm{S},\overline{\mathrm{D}},\overline{\mathrm{T}},t} = (1-p)((1-z_{\mathrm{S}})P^{(6)}_{\mathrm{S},\overline{\mathrm{D}},\mathrm{T},t} + P^{(6)}_{\mathrm{S},\overline{\mathrm{D}},\overline{\mathrm{T}},t})$$

$$P'_{\mathrm{S},\mathrm{D},\overline{\mathrm{T}},t} = (1-p)(1-q_{\mathrm{B}})((1-z_{\mathrm{S}})P^{(6)}_{\mathrm{S},\mathrm{D},\mathrm{T},t} + P^{(6)}_{\mathrm{S},\mathrm{D},\overline{\mathrm{T}},t})$$

$$P'_{\mathrm{L},\overline{\mathrm{D}},\overline{\mathrm{T}},t} = (1-p)(1-q_{\mathrm{L}})((1-z_{\mathrm{L}})P^{(6)}_{\mathrm{L},\overline{\mathrm{D}},\mathrm{T},t} + P^{(6)}_{\mathrm{L},\overline{\mathrm{D}},\overline{\mathrm{T}},t})$$

$$P'_{\mathrm{L},\mathrm{D},\overline{\mathrm{T}},t} = (1-p)(q_{\mathrm{L}}P^{(6)}_{\mathrm{L},\overline{\mathrm{D}},\overline{\mathrm{T}},t} + P^{(6)}_{\mathrm{L},\mathrm{D},\overline{\mathrm{T}},t}) +$$

$$(1-p)(1-z_{\mathrm{L}})(q_{\mathrm{L}}P^{(6)}_{\mathrm{L},\overline{\mathrm{D}},\mathrm{T},t} + P^{(6)}_{\mathrm{L},\mathrm{D},\mathrm{T},t}) +$$

$$(1-p)q_{\mathrm{B}}(P^{(6)}_{\mathrm{S},\mathrm{D},\overline{\mathrm{T}},t} + (1-z_{\mathrm{S}})P^{(6)}_{\mathrm{S},\mathrm{D},\mathrm{T},t})$$

$$P'_{\mathrm{H},\bar{\mathrm{D}},\bar{\mathrm{T}},t} = (1-p)(1-q_{\mathrm{H}})((1-z_{\mathrm{H}})P^{(6)}_{\mathrm{H},\bar{\mathrm{D}},\mathrm{T},t} + P^{(6)}_{\mathrm{H},\bar{\mathrm{D}},\bar{\mathrm{T}},t})$$

$$P'_{\mathrm{H},\mathrm{D},\bar{\mathrm{T}},t} = (1-p)(q_{\mathrm{H}}P^{(6)}_{\mathrm{H},\bar{\mathrm{D}},\bar{\mathrm{T}},t} + P^{(6)}_{\mathrm{H},\mathrm{D},\bar{\mathrm{T}},t}) +$$

$$(1-p)(1-z_{\mathrm{H}})(q_{\mathrm{H}}P^{(6)}_{\mathrm{H},\bar{\mathrm{D}},\mathrm{T},t} + P^{(6)}_{\mathrm{H},\mathrm{D},\mathrm{T},t})$$

$$P'_{\mathrm{I},j,\bar{\mathrm{T}},t} = (1-p)(P^{(6)}_{\mathrm{I},j,\bar{\mathrm{T}},t} + P^{(6)}_{\mathrm{I},j,\mathrm{T},t})$$

$$(1-p)\sum_{i\in\{\mathrm{S,L,H}\}} z_i P^{(6)}_{i,j,T,t}$$

The first two equations state that all occupied burrows with viable treatment are triggered during this movement phase, and only non-triggered and unoccupied burrows keep their non-triggered status, respectively. The density of switching wombats in each state is given by:

$$N_{\mathrm{S}} = p\sum_j (P^{(6)}_{\mathrm{S},j,\bar{\mathrm{T}},t} + (1-z_{\mathrm{S}})P^{(6)}_{\mathrm{S},j,\mathrm{T},t})$$

$$N_{\mathrm{L}} = p\sum_j (P^{(6)}_{\mathrm{L},j,\bar{\mathrm{T}},t} + (1-z_{\mathrm{L}})P^{(6)}_{\mathrm{L},j,\mathrm{T},t})$$

$$N_{\mathrm{H}} = p\sum_j (P^{(6)}_{\mathrm{H},j,\bar{\mathrm{T}},t} + (1-z_{\mathrm{H}})P^{(6)}_{\mathrm{H},j,\mathrm{T},t})$$

$$N_{\mathrm{I}} = p\sum_{j,k} P^{(6)}_{\mathrm{I},j,k,t} + p\sum_{i\in\{\mathrm{S,L,H}\},j} z_i P^{(6)}_{i,j,\mathrm{T},t}.$$

The total density of switchers is denoted $N = \sum_i N_i$. The proportion of burrows in each of the four disease-treatment combinations (i.e., the probability that switchers move to each burrow type) is:

$$\phi_{j,k} = \frac{P'_{\mathrm{U},j,k,t}}{\sum_{j,k} P'_{\mathrm{U},j,k,t}}.$$

After switchers are randomly allocated to burrows the distribution of unoccupied burrows is:

$$P^{(7)}_{\mathrm{U},j,k,t} = P'_{\mathrm{U},j,k,t} - \phi_{j,k}N.$$

and the distribution of occupied burrows is:

$$P^{(7)}_{\mathrm{S},\bar{\mathrm{D}},\bar{\mathrm{T}},t} = P'_{\mathrm{S},\bar{\mathrm{D}},\bar{\mathrm{T}},t} + N_{\mathrm{S}}(\phi_{\bar{\mathrm{D}},\bar{\mathrm{T}}} + \phi_{\bar{\mathrm{D}},\mathrm{T}}(1-z_{\mathrm{S}}))$$

$$P^{(7)}_{\mathrm{S,D,\overline{T},}t} = P^{'}_{\mathrm{S,D,\overline{T},}t} + N_{\mathrm{S}}(1-q_{\mathrm{B}})(\phi_{\mathrm{D,\overline{T}}} + \phi_{\mathrm{D,T}}(1-z_{\mathrm{S}}))$$

$$P^{(7)}_{\mathrm{L,\overline{D},\overline{T},}t} = P^{'}_{\mathrm{L,\overline{D},\overline{T},}t} + N_{\mathrm{L}}(1-q_{\mathrm{L}})(\phi_{\mathrm{\overline{D},\overline{T}}} + \phi_{\mathrm{\overline{D},T}}(1-z_{\mathrm{L}}))$$

$$P^{(7)}_{\mathrm{L,D,\overline{T},}t} = P^{'}_{\mathrm{L,D,\overline{T},}t} + N_{\mathrm{S}}q_{\mathrm{B}}(\phi_{\mathrm{D,\overline{T}}} + \phi_{\mathrm{D,T}}(1-z_{\mathrm{L}})) \;\; +$$

$$N_{\mathrm{L}}(\phi_{\mathrm{\overline{D},\overline{T}}}q_{\mathrm{L}} + \phi_{\mathrm{\overline{D},T}}q_{\mathrm{L}}(1-z_{\mathrm{L}}) \;\; +$$

$$\phi_{\mathrm{D,\overline{T}}} + \phi_{\mathrm{D,T}}(1-z_{\mathrm{L}}))$$

$$P^{(7)}_{\mathrm{H,\overline{D},\overline{T},}t} = P^{'}_{\mathrm{H,\overline{D},\overline{T},}t} + N_{\mathrm{H}}(1-q_{\mathrm{H}})(\phi_{\mathrm{\overline{D},\overline{T}}} + \phi_{\mathrm{\overline{D},T}}(1-z_{\mathrm{H}}))$$

$$P^{(7)}_{\mathrm{H,D,\overline{T},}t} = P^{'}_{\mathrm{H,D,\overline{T},}t} + N_{\mathrm{H}}(\phi_{\mathrm{\overline{D},\overline{T}}}q_{\mathrm{H}} + \phi_{\mathrm{\overline{D},T}}q_{\mathrm{H}}(1-z_{\mathrm{H}}) +$$

$$\phi_{\mathrm{D,\overline{T}}} + \phi_{\mathrm{D,T}}(1-z_{\mathrm{H}}))$$

$$P^{(7)}_{\mathrm{I,\overline{D},\overline{T},}t} = P^{'}_{\mathrm{I,\overline{D},\overline{T},}t} + N_{\mathrm{I}}(\phi_{\mathrm{\overline{D},\overline{T}}} + \phi_{\mathrm{\overline{D},T}}) \;\; +$$

$$\phi_{\mathrm{\overline{D},T}}(z_{\mathrm{S}}N_{\mathrm{S}} + z_{\mathrm{L}}N_{\mathrm{L}} + z_{\mathrm{H}}N_{\mathrm{H}})$$

$$P^{(7)}_{\mathrm{I,D,\overline{T},}t} = P^{'}_{\mathrm{I,D,\overline{T},}t} + N_{\mathrm{I}}(\phi_{\mathrm{D,\overline{T}}} + \phi_{\mathrm{D,T}}) \;\; +$$

$$\phi_{\mathrm{D,T}}(z_{\mathrm{S}}N_{\mathrm{S}} + z_{\mathrm{L}}N_{\mathrm{L}} + z_{\mathrm{H}}N_{\mathrm{H}})$$

Here, in the absence of data suggesting otherwise, we assume that only wombats in the high-disease state are the source of new mite infections in burrows (i.e. $q_{\mathrm{L}} = 0$). We also assume that the disease-state of a wombat does not influence the likelihood that treatment is successfully delivered to wombats upon flap triggering (i.e. $z_{\mathrm{S}} = z_{\mathrm{L}} = z_{\mathrm{H}}$).

IV.III.VIII Wombat independence

On average, it takes τ days for a female to have a single offspring. Here, we assume single offspring are produced every 18 months per female, which implies $\tau = 547.5$ days. Assuming a 50:50 sex-ratio, the density of healthy and infected dispersing young are:

$$b_{\mathrm{U},t} = \frac{1}{2\tau}\sum_{i=\{\mathrm{S,I}\},j,k} P^{(7)}_{i,j,k,t}$$

$$b_{\mathrm{D},t} = \frac{1}{2\tau}\sum_{i=\{\mathrm{L,H}\},j,k} P^{(7)}_{i,j,k,t}$$

The proportion of young dispersers that survive to find an empty burrow is:

$$s_t = \min\{b_{\mathrm{U},t} + b_{\mathrm{D},t}, \sum_{j,k} P^{(7)}_{\mathrm{U},j,k,t}\}/(b_{\mathrm{U},t} + b_{\mathrm{D},t})$$

When dispersing, these young settle in the four unoccupied burrow states with probabilities:

$$\phi_{j,k} = \frac{P^{(7)}_{\mathrm{U},j,k,t}}{\sum_{j,k} P^{(7)}_{\mathrm{U},j,k,t}}.$$

After dispersal, the burrow distribution is:

$$P^{(8)}_{\mathrm{S},\overline{\mathrm{D}},\overline{\mathrm{T}},t} = P^{(7)}_{\mathrm{S},\overline{\mathrm{D}},\overline{\mathrm{T}},t} + s_t b_{\mathrm{U},t}(\phi_{\overline{\mathrm{D}},\overline{\mathrm{T}}} + \phi_{\overline{\mathrm{D}},\mathrm{T}}(1 - z_{\mathrm{S}}))$$

$$P^{(8)}_{\mathrm{S,D},\overline{\mathrm{T}},t} = P^{(7)}_{\mathrm{S,D},\overline{\mathrm{T}},t} + s_t b_{\mathrm{U},t}(1 - q_{\mathrm{B}})(\phi_{\mathrm{D},\overline{\mathrm{T}}} + \phi_{\mathrm{D},\mathrm{T}}(1 - z_{\mathrm{S}}))$$

$$P^{(8)}_{\mathrm{L},\overline{\mathrm{D}},\overline{\mathrm{T}},t} = P^{(7)}_{\mathrm{L},\overline{\mathrm{D}},\overline{\mathrm{T}},t} + s_t b_{\mathrm{D},t}(1 - q_{\mathrm{L}})(\phi_{\overline{\mathrm{D}},\overline{\mathrm{T}}} + \phi_{\overline{\mathrm{D}},\mathrm{T}}(1 - z_{\mathrm{L}}))$$

$$P^{(8)}_{\mathrm{L,D},\overline{\mathrm{T}},t} = P^{(7)}_{\mathrm{L,D},\overline{\mathrm{T}},t} + s_t b_{\mathrm{U},t} q_{\mathrm{B}}(\phi_{\mathrm{D},\overline{\mathrm{T}}} + \phi_{\mathrm{D},\mathrm{T}}(1 - z_{\mathrm{S}})) \; +$$

$$s_t b_{\mathrm{D},t}(\phi_{\mathrm{D},\overline{\mathrm{T}}} + \phi_{\mathrm{D},\mathrm{T}}(1 - z_{\mathrm{L}}) \; +$$

$$q_{\mathrm{L}}\phi_{\overline{\mathrm{D}},\overline{\mathrm{T}}} + q_{\mathrm{L}}\phi_{\overline{\mathrm{D}},\mathrm{T}}(1 - z_{\mathrm{L}}))$$

$$P^{(8)}_{\mathrm{I},\overline{\mathrm{D}},\overline{\mathrm{T}},t} = P^{(7)}_{\mathrm{I},\overline{\mathrm{D}},\overline{\mathrm{T}},t} + s_t \phi_{\overline{\mathrm{D}},\mathrm{T}}(b_{\mathrm{U},t} z_{\mathrm{S}} + b_{\mathrm{D},t} z_{\mathrm{L}})$$

$$P^{(8)}_{\mathrm{I,D},\overline{\mathrm{T}},t} = P^{(7)}_{\mathrm{I,D},\overline{\mathrm{T}},t} + s_t \phi_{\mathrm{D},\mathrm{T}}(b_{\mathrm{U},t} z_{\mathrm{S}} + b_{\mathrm{D},t} z_{\mathrm{L}})$$

$$P^{(8)}_{\mathrm{U},\overline{\mathrm{D}},\overline{\mathrm{T}},t} = P^{(7)}_{\mathrm{U},\overline{\mathrm{D}},\overline{\mathrm{T}},t} - s_t \phi_{\overline{\mathrm{D}},\overline{\mathrm{T}}}(b_{\mathrm{U},t} + b_{\mathrm{D},t})$$

$$P^{(8)}_{\mathrm{U},\bar{\mathrm{D}},\mathrm{T},t} = P^{(7)}_{\mathrm{U},\bar{\mathrm{D}},\mathrm{T},t} - s_t\phi_{\bar{\mathrm{D}},\mathrm{T}}(b_{\mathrm{U},t} + b_{\mathrm{D},t})$$

$$P^{(8)}_{\mathrm{U},\mathrm{D},\bar{\mathrm{T}},t} = P^{(7)}_{\mathrm{U},\mathrm{D},\bar{\mathrm{T}},t} - s_t\phi_{\mathrm{D},\bar{\mathrm{T}}}(b_{\mathrm{U},t} + b_{\mathrm{D},t})$$

$$P^{(8)}_{\mathrm{U},\mathrm{D},\mathrm{T},t} = P^{(7)}_{\mathrm{U},\mathrm{D},\mathrm{T},t} - s_t\phi_{\mathrm{D},\mathrm{T}}(b_{\mathrm{U},t} + b_{\mathrm{D},t})$$

The other states (i.e., non-triggered burrows and burrows occupied by high-diseased wombats) remain unchanged. The possible changes in burrow state that result from this model of independence are depicted in Figure S4.11.

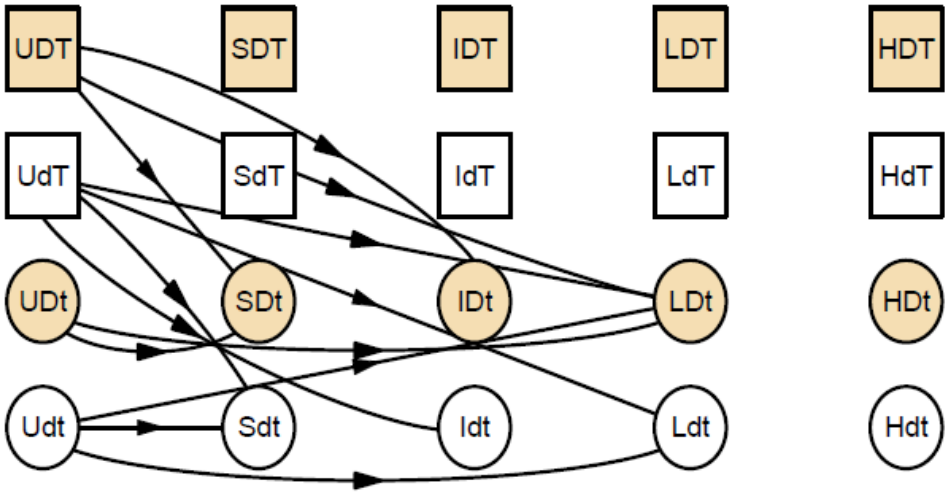

Figure S4.11: Possible changes in burrow states due to offspring independence. Unlike the previous processes, it is not straightforward to associate each transition with a simple formula for the transition probability. Details as to how these probabilities are calculated can be found in the text.

Chapter 5

Chapter 5.0 – Isolation, marine transgression, and translocation of the bare-nosed wombat (*Vombatus ursinus*)

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5.1 Abstract

Island populations can represent genetically distinct and evolutionarily important lineages relative to mainland conspecifics. However, phenotypic divergence of island populations does not necessarily reflect genetic divergence, particularly for lineages inhabiting islands periodically connected during Pleistocene low sea stands. Marine barriers may also not be solely responsible for any divergence that is observed. Here, we investigated genetic divergence among and within the three phenotypically-distinct subspecies of bare-nosed wombats (Vombatus ursinus) in southeast Australia that are presently—but were not historically—isolated by marine barriers. Using genome-wide single nucleotide polymorphisms we identified three genetically distinct groups (mainland Australia, Bass Strait island, Tasmania) corresponding to the recognised subspecies. However, isolation by distance was observed in the Tasmanian population, indicating additional constraints on gene flow can contribute to divergence in the absence of marine barriers, and may also explain genetic structuring among fragmented mainland populations. We additionally confirm origins and quantify the genetic divergence of an island population 46 years after the introduction of 21 individuals from the Vulnerable Bass Strait subspecies. In light of our findings we make recommendations for the maintenance of genetic variation and fitness across the species range.

Key words: Population genetics; Vombatus ursinus; genetic structure; conservation; spatial structure; island biogeography

5.2 Introduction

Islands are frequently the location of populations that can be phenotypically distinguished from those elsewhere (e.g., Harmon and Gibson 2006; Schlotfeldt and Kleindorfer 2006), and contribute to global biodiversity through the effects of isolation on genetic divergence and speciation (Wilson *et al.* 2008). Islands also represent important reservoirs for biodiversity, often removed from threats experienced on other landmasses, such as introduced pests (Short *et al.* 2002). However, island populations can also be of elevated conservation concern, given lower abundances, lack of connectivity, lower genetic diversity, and susceptibility to genetic drift (Frankham 1997). Continental shelf islands are distinctive in this context, experiencing periods of connection to larger landmasses via land bridges during glacial periods when sea levels are low (most recently in the Pleistocene; Burridge 2012). Depending upon the timing, duration, and frequency of these connections, and the nature of intervening habitats, gene flow may have been experienced between lineages occupying presently isolated regions. This raises questions regarding their conservation prioritisation given uncertainty about their history of genetic isolation. Furthermore, phenotypic distinction of lineages on continental shelf islands may also be problematic to interpret if the peripheral geographic setting of these islands confers environmental differences (Mullen *et al.* 2009), in addition to potential influences of island size alone (e.g., dwarfism in island emus; Thomson *et al.* 2018). This is a question of broad conservation interest, as continental shelf islands are common and host high biodiversity, most notably in Southeast Asia (e.g., the entire Malay Archipelago), but also Europe (e.g., England and many islands of the Mediterranean), North America (e.g., Newfoundland), South America (e.g., Falkland Islands), and Australia (e.g., Tasmania) (Burridge 2012).

Historical sea level rise associated with the end of the last glacial maximum potentially played a significant role in the biogeography of south-eastern Australia. This event isolated Tasmania and an array of islands from continental Australia during the flooding of Bass Strait, protecting some populations from causes of extinction that are present on the mainland (e.g., invasive predators; Kinnear et al. 2002), and shaping the population genetic structure of others (Firestone *et al.* 1999, Toon *et al.* 2007). These areas were connected by the Bassian land bridge during the last glacial maximum (LGM) circa 25 kya (Lambeck and Chappell 2001). As sea level rose, the mainland, Tasmania, and intervening islands remained connected through

a western sill until around 17.5 kya and an eastern sill until around 14 kya (Lambeck and Chappell 2001). Many species still occur across these now isolated regions, with Bass Strait and offshore Tasmanian islands exhibiting high species richness per unit area relative to other Australian islands (Burbidge *et al.* 1997), and supporting populations of mammals which are now extinct or declining on mainland Australia (Morris *et al.* 2018). These island populations may represent important genetic lineages and evolutionary legacies that are distinct from the mainland (e.g., platypus; Furlan *et al.* 2012), or may be representative of the mainland genetic pool (e.g., white-bellied sea-eagles; Shephard *et al.* 2005).

Wombats are evolutionarily significant as the largest extant burrowing mammals (Johnson 1998a). The bare-nosed wombat (*Vombatus ursinus*) is a large (up to 50 kg), fossorial marsupial endemic and historically widespread in southeast Australia (mainland and islands, Figure 5.1; Triggs 2009, IUCN 2016). Within this range, there are three recognized allopatric subspecies: southeastern mainland (*V. u. hirsutus;* Perry 1810), Bass Strait islands (*V. u. ursinus;* Shaw 1800), and Tasmanian (*V. u. tasmaniensis;* Spender and Kershaw, 1910) (Jackson 2015). These subspecies are distinguished based on distribution and body size, with mainland individuals being the largest and Flinders Island being the smallest (Tate 1951) – though these distinctions are in need of revisitation in an updated and comprehensive way. Despite being considered "common" – *Vombatus ursinus* Least Concern on IUCN Red List (Taggart *et al.* 2016b) – all three subspecies have experienced range retractions since settlement by Europeans (Figure 5.1), and may support several genetically important, yet isolated populations. Specifically, the range of *V. u. hirsutus* has been fragmented and more than halved, and similar retraction has been observed in *V. u. ursinus,* which now exists only on Flinders Island, having gone extinct on King, Cape Barren, Deal, and Clarke islands (Rounsevell *et al.* 1991). The Tasmanian subspecies exists throughout Tasmania with seemingly stable populations across its range (Figure 5.1; DPIPWE 2017). A growing population also exists on Maria Island (Figure 5.1; Ingram 2015), which may represent the descendants of 21 individuals translocated from Flinders Island (Rounsevell 1989), and hence potentially of conservation significance for *V. u. ursinus*. However, records are inconsistent as to whether *V. ursinus* existed on Maria Island prior to this translocation event (Plomley *et al.* 1990, Rounsevell *et al.* 1991).

Despite range retractions observed in *V. ursinus*, it is still distributed relatively continuously, but with areas of fragmentation in the western and northern edges of the mainland

distribution (IUCN 2016). Assessing genetic structure within subspecies could reveal important biological processes, such as dispersal limitations and barriers to gene flow, that are also relevant for conservation with respect to the maintenance of genetic diversity. Evidence for isolation by distance has been observed for *V. u. hirsutus*, with high levels of population differentiation at larger spatial scales (Banks *et al.* 2002). However, sampling in this study was spatially clumped, and patterns of genetic structure and isolation by distance should be addressed within a continuously sampled region (Rosenberg *et al.* 2005, Bradburd *et al.* 2018). Assessing genetic structure within regions (mainland and Tasmania) also provides a valuable contrast for genetic structuring that may be ascribed to isolation by historical sea level rise.

Here, we utilize genome-wide single nucleotide polymorphisms (SNPs) to (i) quantify the population structure of bare-nosed wombats across their current range in the context of the presently recognised subspecies and their potentially dynamic history of connectivity, (ii) document within region genetic variation to assess gene flow within a continuously distributed and sampled subspecies (*V. u. tasmaniensis*), and (iii) assess the genetic provenance of the Maria Island population with respect to conservation genetic resources of *V. u. ursinus*. Discovery of genetically distinct populations across the wombat range will assist in determining spatial units that warrant independent management and support ongoing conservation planning for this Australian marsupial.

5.3 Methods

5.3.1 Sampling locations and tissue collection

A total of 234 bare-nosed wombat tissue samples was collected from 1999–2000 and 2014–2017, from the Australian mainland (*V. u. hirsutus*; n=84), Bass Strait islands (Flinders Island; *V. u. ursinus*.; n=10), Tasmania (*V. u. tasmaniensis*, n=131), and Maria Island (subspecies uncertain; n=9) (Figure 5.1). Tissue samples were collected post-mortem (road-killed) or by live capture (via mesh nets or cage traps). Tissue was collected from the ear (central pinna) using a sterile 3mm biopsy punch (disposable biopsy punch, Kai Medical) and stored in 70% ethanol at -20˚C until DNA extraction.

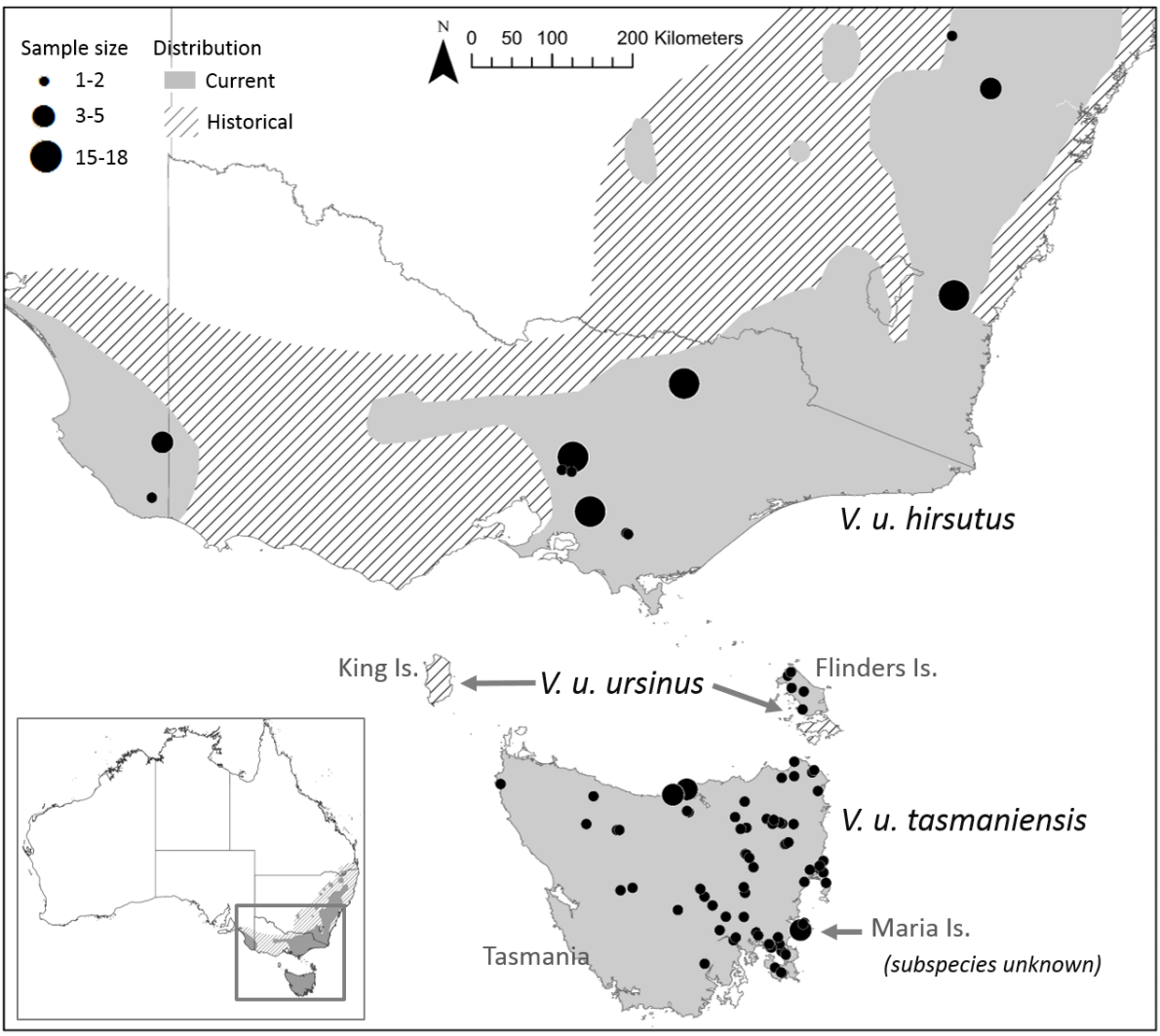


Figure 5.1. The bare-nosed wombat distribution across Australia. Sampling locations and sample size are indicated by the circles (see Supplementary Material I for location coordinates). Spatial data for the current distribution accessed from the International Union for Conservation of Nature.

5.3.2 SNP discovery and filtering

High molecular weight DNA samples (n=176), representative of the bare-nosed wombat distribution, were sent to Diversity Arrays Technology Pty Ltd (DArT), Canberra, Australia for DArTseq analysis. DArTseq utilizes complexity reduction (restriction enzymes *Pst*I and compliment, retained by DArT) and next-generation sequencing methodologies to produce genome-wide single nucleotide polymorphisms (Sansaloni *et al.* 2011, Kilian *et al.* 2012). A total of 28,081 SNPs was identified for *V. ursinus*. SNPs were filtered using the following exclusion criteria: reproducibility (<95%), missing data per locus (>20%), missing data per individual (>10%), secondaries (if multiple SNPs fall on the same sequence, removed the SNP with the lower read count average), minor allele frequencies (≤0.05), mean read depth per sample (<8), and heterozygosity (>0.5). Outlier SNPs identified according to both pcadapt (Luu *et al.* 2017) and sNMF (Frichot and François 2015) were removed. Deviation from Hardy-Weinberg Equilibrium (HWE) was assessed for three sampling regions in *Genepop* (Rousset 2008): Tasmania, Maria and Flinders islands, and one mainland location (central Victoria). SNPs that were out of HWE in two or more of these sampling regions were removed from the dataset (n=372). This approach was taken to reduce the risk of mis-identifying SNPs as out of HWE that are truly reflective of genetic structure (see Section 2.3 for comparative analyses performed including these SNPs). Filtering resulted in a total of 9,064 SNPs for 162 individuals (mainland, n=76; Flinders, n=6; Tasmania, n=74; Maria Island, n=6; Supplementary Material I and II).

5.3.3 Diversity estimates and population structure

Heterozygosity, allelic richness, and F_{ST} were estimated using the R packages *diveRsity* (Keenan *et al.* 2013) and *strataG* (Archer *et al.* 2017). Population structure was explored using a combination of multivariate and Bayesian methodologies. We focused on understanding structure at two different geographic scales: (1) among the three bare-nosed wombat subspecies, and (2) within the Tasmanian subspecies only, to reveal fine-scale structure across a continuous sampling range. In each case, structure was assessed visually using principal component analyses (PCA, package adegenet V2.0.1; Jombart 2008) and Bayesian cluster analysis (fastSTRUCTURE; Raj *et al.* 2014). All fastSTRUCTURE runs used a simple prior with cross validation (cv=10) and explored K=1−10 clusters. The optimal K range was determined

using fastSTRUCTURE algorithms. PCA and fastSTRUCTURE were also performed including SNPs that violated our HWE filtering criterion for comparative purposes (Supplementary Material III).

No additional structure analyses (beyond PCA and fastSTRUCTURE) were performed for the mainland region given the discrete spatial sample distribution and potential for false inference of genetic breaks if isolation by distance operates (Serre and Pääbo 2004, Bradburd *et al.* 2018). However, further estimates of genetic diversity and differentiation were performed for the discrete populations located across the mainland (Supplementary Material IV). In Tasmania, where sampling was more continuous, a spatial Principal Components Analysis (sPCA, package *adespatial*, Dray *et al.* 2018). was performed. sPCA incorporates both genetic variation and spatial autocorrelation (spatial weighting matrices) to explain observed patterns (Jombart *et al.* 2008). A Gabriel's graph was employed as the connection network and sPCA scores were visually represented using the R package *ade4* (Dray and Dufour 2007).

To complement the sPCA, we investigated isolation by distance in Tasmania by employing a redundancy analysis (RDA) following the methodology of Meirmans (2015). The RDA was performed as an individual – rather than population – based analysis, whereby the dependent variable was the allele count per locus per individual, and the independent variable was a set of spatial polynomials derived from geographic coordinates. It is important to note that potential landscape inhibitors to movement (e.g., lakes and rivers) are not considered by this approach. The RDA was performed in R using the package *VEGAN* (Oksanen *et al.* 2018).

5.4 Results

5.4.1 Diversity estimates

Diversity estimates are described in Table 5.1. Eastern mainland locations (Victoria and New South Wales sites) had the highest allelic richness and observed heterozygosity (Ar=1.56–1.60, H_o=0.19–0.21), followed by Tasmania (Ar=1.52, H_o=0.18), and Maria and Flinders islands (Ar=1.35–1.39, H_o=0.15–0.16). The western mainland (South Australian location) had the lowest genetic diversity (Ar=1.29, H_o=0.11).

Table 5.1. Summary statistics for genome-wide SNP loci (n=9064). See Figure 5.2A for locations.

Region	N	N_l	Ar	H_o	H_e
South Australia (SA)	5	4.74	1.29	0.11	0.14
Central Victoria (cVIC)	34	33.28	1.60	0.21	0.24
Eastern Victoria (eVIC)	15	14.59	1.57	0.20	0.23
New South Wales (NSW)	22	21.34	1.56	0.19	0.23
All Mainland	*76*	*73.96*	*1.76*	*0.19*	*0.25*
Flinders Is (FI)	6	5.85	1.39	0.15	0.16
Maria Is (MI)	6	5.80	1.35	0.16	0.15
Flinders and Maria Islands	*12*	*11.65*	*1.46*	*0.15*	*0.17*
Tasmania (TAS)	74	71.87	1.52	0.18	0.21

Number of individuals (N), mean number of individuals typed per locus (N_l), mean allelic richness (Ar), mean observed heterozygosity (Ho), and mean expected heterozygosity (He).

Table 5.2. Pairwise F_{ST} among sampling regions derived from SNPs (left) and corresponding *P*-values (right; corrected using the Benjamini-Hochberg method). All comparisons were significant (≤0.01). Among region comparisons are in bold. See Figure 5.2A for locations.

		Mainland				Islands		Tasmania	All Mainland	Flinders & Maria
		South Australia	Central Victoria	Eastern Victoria	New South Wales	Flinders Is.	Maria Is.			
Mainland	South Australia (SA)	-	0.009	0.009	0.009	0.010	0.010	0.009	-	-
	Central Victoria (cVIC)	0.212	-	0.009	0.009	0.009	0.009	0.009	-	-
	Eastern Victoria (eVIC)	0.229	0.074	-	0.009	0.009	0.009	0.009	-	-
	New South Wales (NSW)	0.248	0.107	0.079	-	0.009	0.009	0.009	-	-
Islands	Flinders Is. (FI)	0.426	0.264	0.267	0.276	-	0.010	0.009	-	-
	Maria Is. (MI)	0.458	0.284	0.291	0.298	0.047	-	0.009	-	-
	Tasmania (TAS)	0.416	0.351	0.352	0.361	0.317	0.334	-	**0.009**	**0.009**
	All Mainland	-	-	-	-	-	-	**0.320**	-	**0.009**
	Flinders & Maria	-	-	-	-	-	-	**0.325**	**0.241**	-

5.4.2 Population structure

Pairwise fixation indices estimated among regions (pooled locations: mainland, Flinders and Maria islands, and Tasmania) ranged from 0.24–0.33 (Table 5.2), and all were significant ($P \leq 0.01$) after correction for false discovery rates. The mainland was less differentiated from Flinders and Maria islands (F_{ST}=0.24) than it was from Tasmania (F_{ST}=0.32), and Tasmania was most differentiated from the Flinders and Maria islands (F_{ST}=0.33). Within the mainland, central Victoria (cVIC), eastern Victoria (eVIC), and New South Wales (NSW) had lower population differentiation (F_{ST}=0.07–0.11), but experienced higher differentiation from South Australia (SA; F_{ST}=0.21–0.25). Differentiation was also assessed at the population level within mainland groupings (Supplementary Material IV). Flinders Island (FI) and Maria Island (MI) showed very little genetic differentiation (F_{ST}= 0.05), and their differentiation from other populations were similar (Table 5.2).

Principal component analysis revealed three non-overlapping clusters, with PC1 and PC2 explaining 25.9% and 4.5% of the variance, respectively. The groupings were as follows: (1) all Tasmanian individuals, (2) Maria Island and Flinders Island individuals, and (3) all mainland individuals. The fastSTRUCTURE analysis produced results consistent with the PCA when K was set to 3 (Figure 5.2, Supplementary Material V), with assignment plots corresponding to the groups from the PCA. Additional structure was assessed for K=5−6, the K range suggested by fastSTRUCTURE. K=5−6 consistently grouped all Tasmanian samples together and Maria and Flinders Islands samples together, with further sub-structuring suggested among mainland locations (Figure 5.2, Supplementary Material VI). PCAs and fastSTRUCTURE were also performed independently for the mainland, Flinders and Maria Islands, and Tasmania (Figure 5.3A; Supplementary Material VII, VIII, and IX), but no additional structure was only observed in Tasmania.

fastSTRUCTURE suggested a K range from 1−3 for Tasmania (Figure 5.3A), though most individuals were assigned to the same cluster. fastSTRUCTURE ancestry proportions clustered eight individuals – predominately from north-central Tasmania – into a separate cluster when K=3 (each having >99% of their ancestry assigned to this cluster). One of the eight individuals in the separate cluster was spatially discordant (from the east). sPCA revealed significant global structure in Tasmania ($P<0.01$), but no local structure (P=0.95). Individuals were genetically

similar to those sampled adjacently, with the exception of east-west comparisons across north-central Tasmania (PC1 34.8%; Figure 5.3B). Principal component 2 (PC2 30.7%; Figure 5.3C) showed differentiation of south-eastern Tasmania (Tasman Peninsula and surroundings). RDA revealed significant correlation between genetic variation and geographic coordinates of samples (17.6% of the genetic variation explained by geographic coordinates, *P*=0.001).

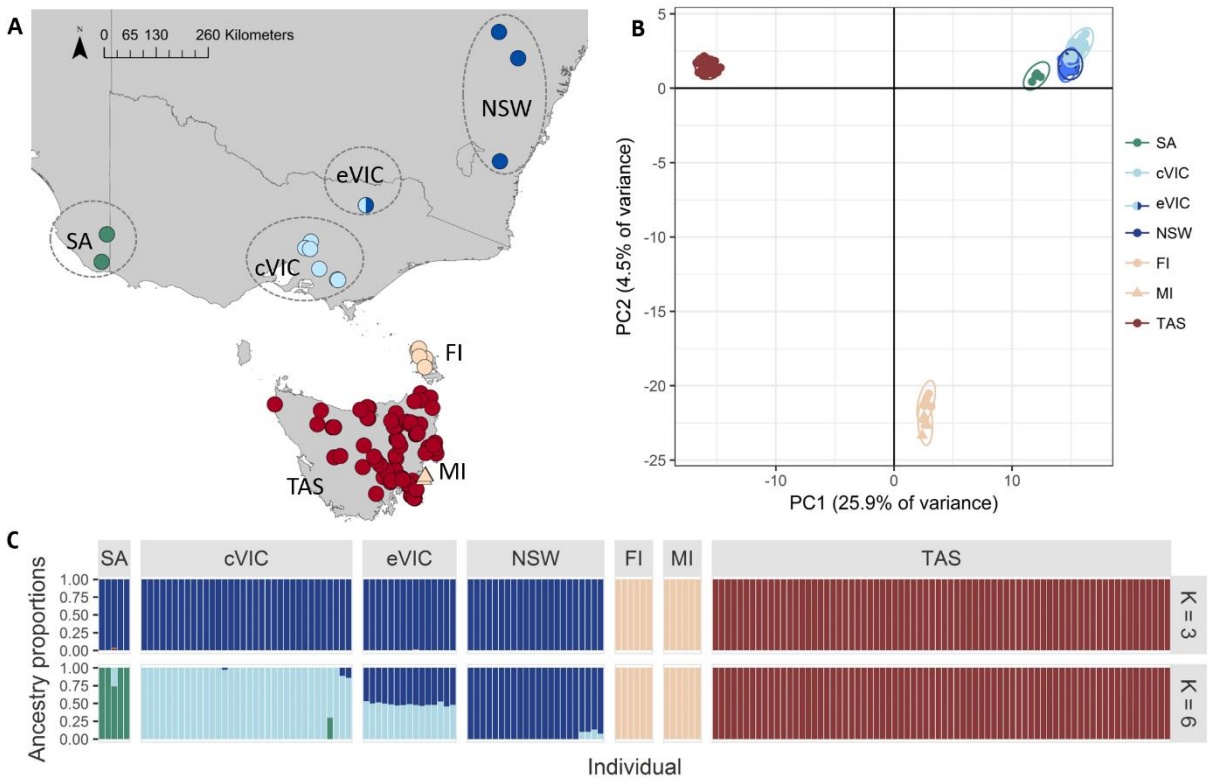


Figure 5.2. Genetic structuring of bare-nosed wombats. Sample geographic locations (A) with colours corresponding to the results from PCA (B) and fastSTRUCTURE (C). Sampling location codes are: South Australia (SA), central Victoria (cVIC), eastern Victoria (eVIC), New South Wales (NSW), Tasmania (TAS), Flinders Island (FI) and Maria Island (MI). PCA plot includes a 99% confidence ellipse for each location.

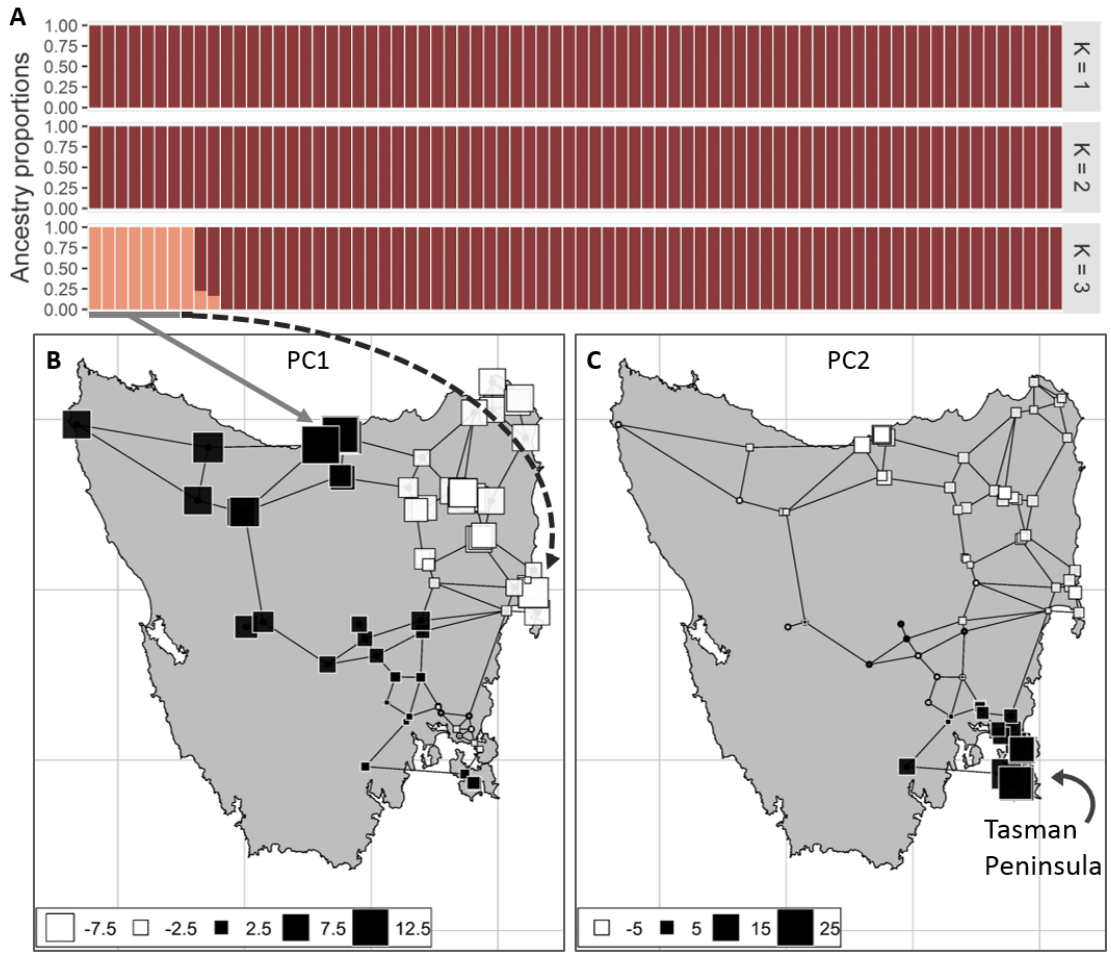


Figure 5.3. fastSTRUCTURE (A) and sPCA (B, C) results for Tasmanian individuals. Spatial mapping of the principal components 1 (B) and 2 (C) of the sPCA visually represents genetic differentiation proportional to difference in square size and shade. Arrows designate where the eight individuals assigned to the separate cluster (>99% ancestry proportion; A) are geographically located.

5.5 Discussion

5.5.1 Genetic differentiation among *V. ursinus* subspecies

Designations of *Vombatus ursinus* subspecies originated in the mid-1800s and early 1900s when differences in body and skull size were observed in the geographically separated groups. The mainland subspecies was described as the largest and Flinders Island *V. ursinus* were the smallest (Tate 1951). While body size is often a distinguishable feature between island populations and their mainland conspecifics (Lomolino 1985), observed differences between groups does not necessarily denote genetic divergence (Thomson *et al.* 2018). Here, our genome-wide SNP analyses identified three genetic groups of *V. ursinus* that correspond to the presently recognised subspecies: mainland, *V. u. hirsutus*; Bass Strait, *V. u. ursinus*; and Tasmania, *V. u. tasmaniensis*.

Continental islands of Australia have been geographically separated from the mainland by sea level rise for ~6–17 thousand years (Coller 2007), and genetic differentiation among island and mainland populations has been observed in several instances (e.g., Kangaroo Island, Morris *et al.* 2018). Several species exhibit significant genetic divergence across Bass Strait: Bennett's wallaby, *Macropus rufogriseus* (Le Page *et al.* 2001); spotted-tailed quoll, *Dasyurus maculatus* (Firestone *et al.* 1999); and platypus, *Ornithorhynchus anatinus* (Furlan *et al.* 2010, Gongora *et al.* 2012). Lowered genetic diversity has also been observed when compared to mainland lineages (platypus, *O. anatinus*; Furlan *et al.* 2012), which is a pattern commonly observed in island populations (Frankham 1997). However, marine barriers have not influenced genetic structure for all species, such as the grey kangaroo, *Macropus giganteus* (Zenger *et al.* 2003), wedge-tailed eagle, *Aquila audax* (Burridge et al. 2013), and white-bellied sea eagle, *Haliaeetus leucogaster* (Shephard et al. 2005). Genetic structure (or lack-there-of) during comparisons of mainland and continental island populations may be influenced by several factors, including species dispersal capability and the environmental suitability of the land bridge.

It is evident that marine barriers have impacted the genetic structure of bare-nosed wombats over and above that observed in their absence (e.g., divergence observed among subspecies compared to within). However, the genetic divergence of these populations does not immediately align with our current understanding of historical marine isolation. Specifically, the reconstruction of the southern coastline of Australia suggests that the flooding of the

Bassian Plain separated the mainland from both Tasmania and Flinders Island first, while a land bridge still connected Tasmania and Flinders Island for an additional ~5–7k years (Lambeck and Chappell 2001, Coller 2007). However, mainland and Flinders Island subspecies exhibit less genetic distinction from each other than when compared to Tasmania. Two plausible explanations exist for these patterns. First, it is possible that gene flow across the Bassian Plain was influenced by factors other than sea level, and that despite being physically connected, geneflow was not achieved between Tasmania and Flinders Island following their isolation from the mainland. Second, F_{ST} is influenced by both population size and gene flow (Meirmans and Hedrick 2011), and thus a combination of our sample sizes and the population sizes may have influenced the genetic divergence observed. Therefore, estimates of divergence time are required to assess whether marine barriers initiated or reinforced the isolation of these populations (e.g., Burridge *et al*. 2013), and should be pursued in future analyses.

5.5.2 Genetic structure within subspecies

Within Tasmania, where sampling was more continuous, there was evidence for isolation by distance. While bare-nosed wombats are capable of dispersal across varied landscapes (as their distribution suggests), they exhibit relatively small home ranges (on average 17.7 hectares; Evans 2008). Furthermore, dispersal is female-biased in all wombat species (Johnson and Crossman 1991, Banks *et al.* 2002, Walker *et al.* 2008), and though the extent of these movements is not well-understood, there is molecular and tracking based evidence that suggests they are of short distances (100–3000m). These short-distance dispersal behaviours may provide some explanation for the isolation by distance observed within Tasmania. The exception to this pattern was observed in east-west comparisons in the north-central region of Tasmania, where geographically close individuals were genetically dissimilar, in a manner akin to a 'ring species' (Irwin *et al.* 2001). This likely reflects long-term barriers to gene flow present in this region, such as the Tamar River, with more recent (and likely weaker) impact from urbanisation (the city of Launceston, the second largest city in Tasmania) and degraded landscapes (agricultural lands). Future research should investigate landscape features at finer scales to disentangle the potential contributors to this genetic break.

While most Tasmanian individuals were assigned to the same population cluster (n=66, >90% ancestry assigned to the same cluster), it is worth noting that eight individuals were assigned (>99% ancestry) to a separate population cluster. Seven of these individuals were from the Tamar Valley region (north-central Tasmania), specifically Narawntapu National Park and Greens Beach area. These locations are geographically close (<20km) and well sampled in consecutive years due to research conducted in the area (Martin *et al.* 2018a). Thus, this genetic cluster may reflect sampling of close relatives. The eighth individual assigned to this cluster was geographically distant and may reflect a translocation event resulting from wildlife rescue. Current wombat rehabilitation guidelines suggest a release site near the individual's capture location, but this is not always possible, and thus it is not uncommon that an individual is raised or rehabilitated and released in a different location. This individual was not distinguished in the sPCA results: the discrepancy between analyses may reflect a lack of spatial information incorporated into fastSTRUCTURE, and reveals potential limitations in identifying migrant (or translocated) individuals using sPCA.

Though our mainland sampling was more spatially discrete, which places constraints on the interpretation of genetic structuring (Bradburd *et al.* 2018), we found high genetic differentiation within *V. u. hirsutus* specifically against the South Australian samples (SA). This longitudinal pattern of genetic differentiation is consistent with previous studies of *V. u. hirsutus*, using microsatellite loci (Banks *et al.* 2002). This may be reflective of the recent fragmentation across the western range of *V. u. hirsutus* (IUCN 2016), as the eastern mainland are less differentiated over comparable spatial scales. Further, the SA population is likely smaller, and thus more susceptible to genetic drift (Frankham 1996). These patterns may also be observed in the fragmented range in southern Queensland and northern New South Wales; however, samples from these regions were absent from our analyses. Finer spatial sampling across the mainland is required to determine factors responsible for genetic structuring in this region.

5.5.3 *V. u. ursinus* on Maria Island

Bare-nosed wombats have been subjected to considerable human interference across the Bass Strait islands, becoming extinct on King, Cape Barren, Flinders, Deal, and Clarke islands. Given

this history, *V. u. ursinus* was listed as Vulnerable in 2008 under the *Environment Protection and Biodiversity Conservation Act 1999* (Commonwealth EPBC). However, we reveal a second population of *V. u. ursinus* located on Maria Island. Following the translocation event of 1971, wombats on Maria Island were considered rare (Rounsevell *et al.* 1991). However, the present population is prolific and has experienced growth over the last decade (Ingram 2015). The Maria Island population has two implications for the conservation of *V. u. ursinus*: (i) it represents security for the future of *V. u. ursinus*, and (ii) indicates the potential ease at which *V. u. ursinus* could be re-introduced to Bass Strait islands.

We observe no genetic signature of multiple *V. ursinus* subspecies in the Maria Island population, suggesting either (i) *V. u. tasmaniensis* was present at the time of translocation but no genetic signature has been retained to present, or (ii) this lineage was not present at the time of translocation. If wombats were already present at the time of the Flinders translocation event, their low abundance may have reflected inbreeding depression (Frankham 2010), and the translocation may have constituted a genetic rescue event (Whiteley *et al.* 2015, Frankham *et al.* 2016). Despite founding by only 21 individuals, genetic diversity in the Maria Island population was comparable to that of Flinders Island. This is supported by similar estimates of allelic richness and the low pairwise fixation index. Therefore, this translocation event may have captured most of the genetic variation on Flinders Island. However, it is possible that Flinders Island has experienced a loss in diversity since the translocation event, resulting in similar diversity estimates to Maria Island, which are low compared to Tasmania and the mainland. Furthermore, the Flinders-Maria fixation index is significantly greater than zero and may indicate important genetic differentiation, or in this case, may be reflective of a founder effect or genetic drift (Weeks *et al.* 2016). The lowered genetic diversity (allelic richness) observed in both Maria and Flinders islands populations, and to a lesser extent in Tasmania, is typical of island populations (Frankham 1997), but may require management action if low fitness is observed in the future (i.e., genetic rescue; Frankham 2015, Whiteley *et al.* 2015).

5.5.4 Applied evolutionary management

There is ongoing debate regarding the genetic identification of intraspecific units warranting independent conservation (Coates *et al.* 2018). Given the identification of three genetically and

phenotypically distinct wombat lineages across geographically (and reproductively) isolated regions, it may be appealing to consider the subspecies separately for management purposes, as legislation often considers subspecies as separate entities for conservation (Coates *et al.* 2018). Significant genetic divergence was also observed among recently fragmented mainland wombat populations. However, neutral genetic divergence among populations may not necessarily reflect adaptive differences (Crandall *et al.* 2000, Coates *et al.* 2018, Ralls *et al.* 2018), and could instead reflect the action of genetic drift during population declines, concomitantly reducing genetic diversity. Under such circumstances, management to maintain genetic distinctiveness of populations could increase their extinction risk if they suffer from low fitness, potentially reflecting inbreeding depression or genetic load (Hedrick and Fredrickson 2010, Weeks *et al.* 2016, Ralls *et al.* 2018). Research on bare-nosed wombats to assess fitness and adaptive distinction has been insufficient, although dramatic population declines have been observed in some areas (e.g., in response to novel pathogens; Martin *et al.* 2018a). As additional resources become available (i.e., the annotation of the wombat genome), questions regarding adaptive distinction can also be investigated more thoroughly (Pardo-Diaz *et al.* 2015). Regardless, if fitness is low, there are potential benefits through the incorporation of genetic variation from other populations ("genetic rescue"; Frankham 2015, Ralls *et al.* 2018). However, controlled crosses need first be conducted to assess potential fitness benefits, and the risk of outbreeding depression (although these appear overstated, generally; Frankham et al., 2011). The Bass Strait islands previously harbouring *V. u. ursinus* provide an ideal opportunity to both establish additional insurance populations of pure *V. u. ursinus*, and also to test the potential fitness benefits of crosses within and between subspecies, if indeed natural populations are ascertained to be threatened by low fitness.

I. Bare-nosed wombat sample location details (total of 162; sample size per location, N).

State	N	Latitude	Longitude	Sample ID
Flinders Is	1	-39.995	148.060	F4
Flinders Is	1	-39.821	147.883	Reg1
Flinders Is	1	-39.819	147.882	Reg2
Flinders Is	1	-39.778	147.916	Reg3
Flinders Is	1	-39.955	147.925	Reg4
Flinders Is	1	-40.195	148.047	Reg6
Maria Is	2	-42.581	148.066	M1_ms
Maria Is	3	-42.661	148.024	MX_ms
Maria Is	1	-42.593	148.052	M9_ms
New South Wales	18	-35.571	149.739	Vur_GS_X
New South Wales	3	-33.255	150.148	Vur_JO_X
New South Wales	1	-32.667	149.717	Vur_SB_004
South Australia	1	-37.828	140.780	Vur_SB_021
South Australia	4	-37.211	140.898	Vur_TC_X
Tasmania	1	-41.106	146.791	391
Tasmania	3	-41.090	146.754	397
Tasmania	1	-42.898	147.813	Am11
Tasmania	1	-41.810	147.409	Am12
Tasmania	1	-42.684	147.530	Bek
Tasmania	1	-42.662	147.122	BN01
Tasmania	1	-41.818	147.422	Cam1
Tasmania	1	-41.343	146.775	CB1
Tasmania	1	-42.217	146.014	CB12
Tasmania	1	-41.476	145.632	CB13
Tasmania	1	-42.437	146.654	CB15
Tasmania	1	-42.189	146.148	CB16
Tasmania	1	-41.518	147.419	CB17
Tasmania	1	-41.531	147.355	CB18_ms
Tasmania	1	-42.245	147.405	CB2
Tasmania	1	-43.081	147.739	CB3
Tasmania	1	-42.817	147.789	CB4
Tasmania	1	-42.514	147.391	CB5
Tasmania	1	-42.688	147.529	CB6
Tasmania	1	-42.741	147.772	CB8
Tasmania	1	-43.140	147.820	CH004
Tasmania	1	-43.134	147.807	Ch006
Tasmania	1	-42.857	147.697	CR1
Tasmania	1	-42.134	148.309	FNP1
Tasmania	1	-41.979	148.239	FNP2
Tasmania	1	-41.888	148.279	FNP3_ms
Tasmania	2	-42.017	148.279	FNPX

Tasmania	1	-41.544	145.974	Grob1
Tasmania	1	-41.543	146.001	Grob7
Tasmania	1	-42.818	147.672	H2
Tasmania	1	-42.941	147.862	Jbog1
Tasmania	1	-42.935	147.859	Jbog2
Tasmania	1	-41.226	147.400	KS10
Tasmania	1	-41.703	147.851	KS2
Tasmania	1	-41.470	147.819	KS3
Tasmania	1	-41.458	147.792	KS4
Tasmania	1	-41.419	147.647	KS5
Tasmania	1	-41.330	146.757	KS7
Tasmania	1	-43.037	146.953	KS9
Tasmania	1	-40.780	147.955	MtW1
Tasmania	1	-40.899	148.154	MtW2
Tasmania	1	-42.775	147.277	Old B
Tasmania	1	-41.165	145.712	RKW003
Tasmania	1	-41.988	148.129	SC1
Tasmania	1	-42.512	147.190	SC10
Tasmania	1	-40.875	148.175	SC13
Tasmania	1	-40.942	147.953	SC14
Tasmania	1	-42.123	148.067	SC15
Tasmania	1	-40.960	147.814	SC16
Tasmania	1	-41.107	148.218	SC17
Tasmania	1	-42.386	147.041	SC2
Tasmania	1	-42.744	147.301	SC5
Tasmania	1	-42.181	147.390	SC6
Tasmania	1	-42.287	146.951	SC7
Tasmania	1	-41.029	144.675	SC8
Tasmania	1	-42.722	147.551	Scabies_ms
Tasmania	1	-42.201	146.905	TL05
Tasmania	1	-41.474	147.711	TL06
Tasmania	1	-41.399	147.293	TL09
Tasmania	1	-41.430	147.725	TL1
Tasmania	1	-41.959	147.498	TL12
Tasmania	1	-41.477	147.946	TL2
Tasmania	2	-41.430	147.725	TLX
Tasmania	1	-41.696	147.862	TT1
Tasmania	1	-41.681	147.889	TT2
Tasmania	1	-41.944	148.236	TT3
Tasmania	3	-41.150	146.599	W00X
Tasmania	1	-41.854	147.452	WOM
Victoria	15	-37.376	145.484	Vur_LS_X
Victoria	15	-36.556	146.724	Vur_MW_1
Victoria	15	-37.986	145.675	Vur_SB_006
Victoria	1	-37.518	145.359	Vur_SB_023
Victoria	1	-37.538	145.468	Vur_SB_024

Victoria	1	-38.225	146.078	WW01
Victoria	1	-38.237	146.098	WW02

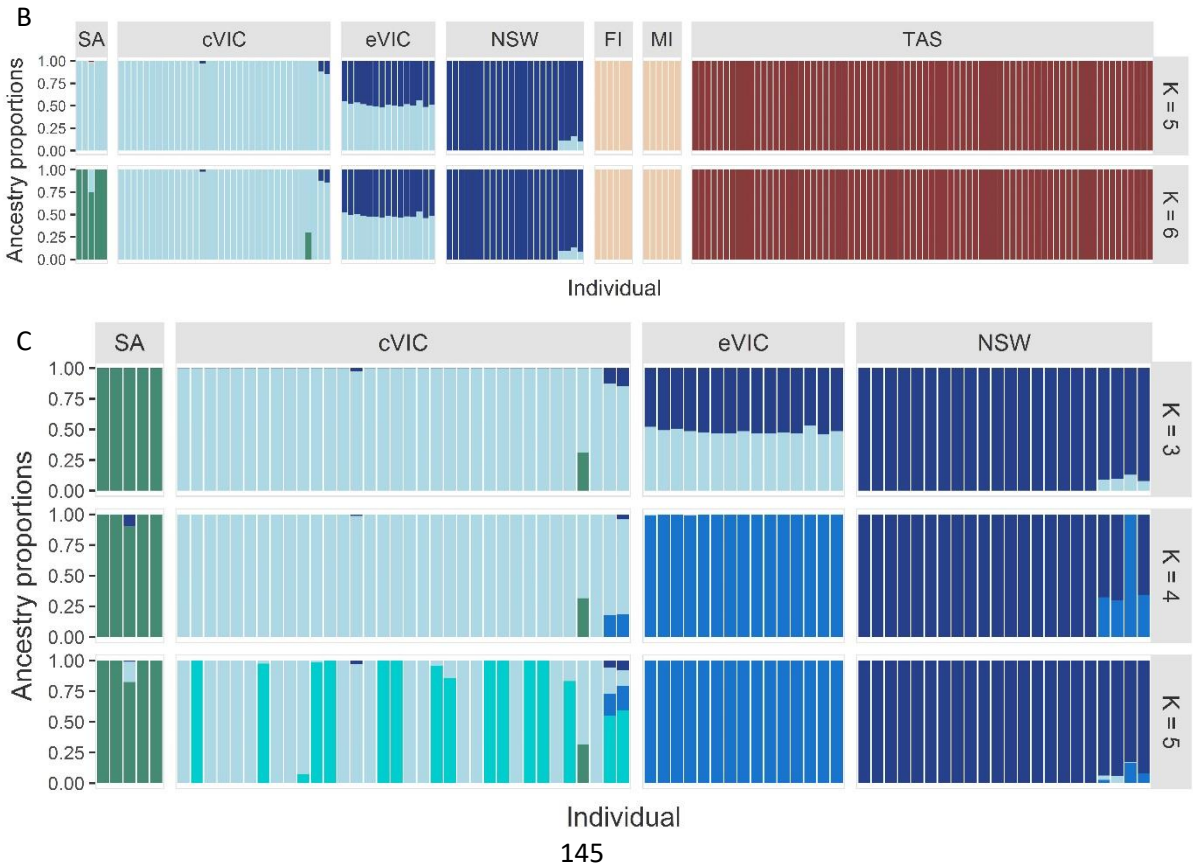
II. Number of single nucleotide polymorphisms and individuals retained after applying each filtering step for the bare-nosed wombat (*Vombatus ursinus*) DArT data set.

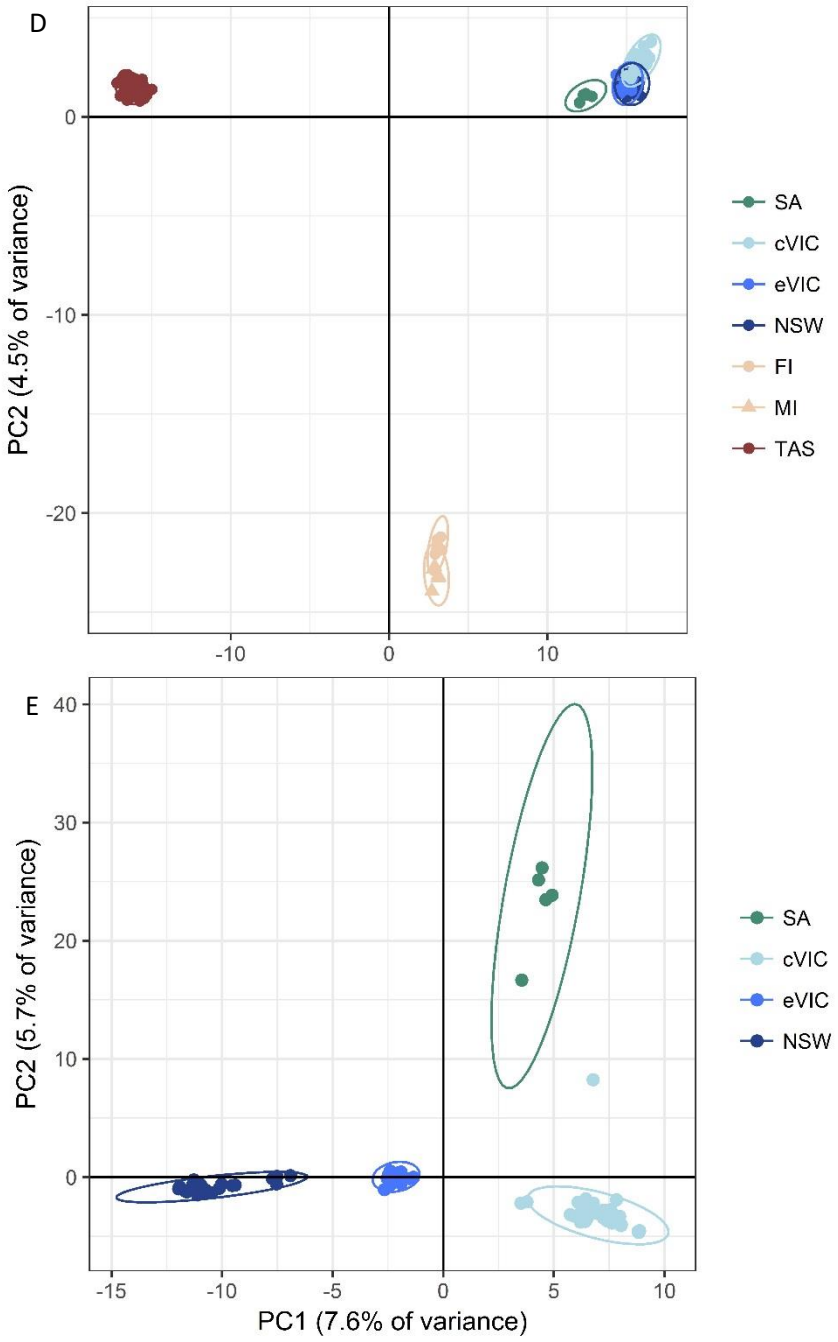
Exclusion criteria (filtering step)	Individual count	SNP Count
Raw SNP data set	165	28,081
(1) Reproducibility (<95%)	165	27,475
(2) Missing data per locus (>20%)	165	22,619
(3) Secondaries (If two SNPs fall on the same fragment, the SNP with the lower read count average is removed)	165	18,329
(4) Missing data per individual (>10%) (Loci that become monomorphic due to excluded individuals are also removed)	162	18,311
(5) Minor allele frequency (≤0.05)	162	10,760
(6) Read depth (coverage depth <8)	162	9,816
(7) Heterozygosity (>0.5)	162	9,778
(8) Outliers identified by both PCADAPT and SNMF (significance < 0.05) PCADAPT identified 1,034 SNPs sNMF identified 1,029 SNPs	162	9,436
(9) Hardy-Weinberg dis-equilibrium in ≥2 locations (GENEPOP)	162	9,064

III. To explore the impact of filtering SNPs based on Hardy-Weinberg Equilibrium criteria, fastSTRUCTURE analysis and PCAs were performed including the 372 SNPs. fastSTRUCTURE results were largely the same (A), with the exception of the most likely number of clusters for the mainland. Assignment plots were created for the all samples (B) and mainland only (C). PCA results for all samples (D) and mainland only (E) are also shown.

A. Results of fastSTRUCTURE for K=1−10 for different sampling regions.

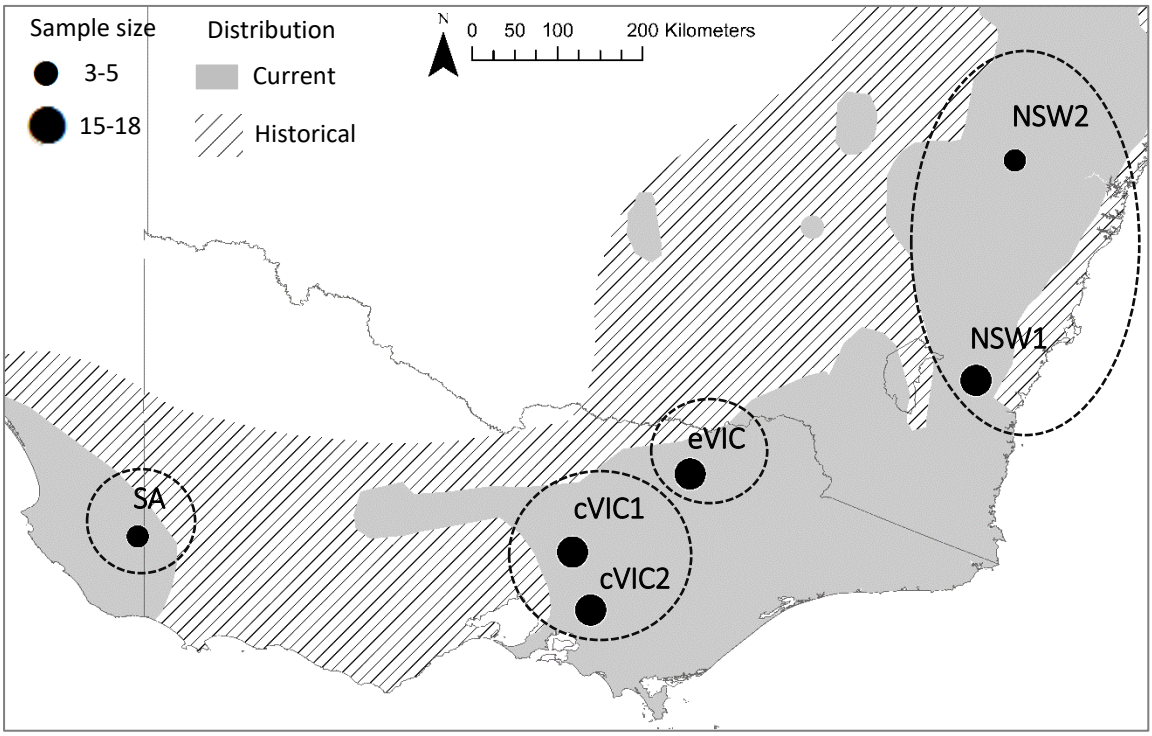
K	Sampling region			
	All	Mainland only	Tasmania only	Maria & Flinders
1	-0.903080	-0.808204	**-0.680214**	**-0.715486**
2	-0.757455	-0.800275	-0.680783	-0.716102
3	-0.742196	**-0.796945**	-0.697503	-0.716342
4	-0.738415	-0.803683	-0.696174	-0.716474
5	-0.738475	-0.812736	-0.712313	-0.786648
6	**-0.736798**	-0.819058	-0.681264	-0.716619
7	-0.736846	-0.812829	-0.696735	-0.716665
8	-0.736886	-0.811328	-0.681340	-0.716701
9	-0.738654	-0.808638	-0.681367	-0.716731
10	-0.736942	-0.812960	-0.681390	-0.716756
K based on model complexity	6	3	1	1
K based on model components	5	5	1	1




D
0
-10
-20
PC2 (4.5% of variance)
-10
0
10
SA
cVIC
eVIC
NSW
FI
MI
TAS
E
40
30
20
10
0
PC2 (5.7% of variance)
-15
-10
-5
0
5
10
PC1 (7.6% of variance)
SA
cVIC
eVIC
NSW

IV. Estimates of diversity and differentiation for mainland populations. 'Populations' were sites with ≥3 individuals sampled in the same location (n=6, labels upper right; A). Allelic diversity (B) and pairwise F_{ST} (C) were estimated for each population using the same methods described in the main text. An hierarchical analysis of molecular variance (AMOVA, package poppr; Kamvar *et al.* 2015) was performed across population and regional (SA, cVIC, eVIC, and NSW; black dotted circles) levels (D).

A



B. Summary statistics for genome-wide SNP loci (n=9064) for the six mainland populations.

Region	N	N_l	Ar	H_o	H_e
South Australia (SA)	4	3.81	1.24	-	0.14
Central Victoria 1 (cVIC1)	15	14.67	1.48	0.21	0.23
Central Victoria 2 (cVIC2)	15	14.59	1.48	0.20	0.23
Eastern Victoria (eVIC)	15	14.69	1.47	0.20	0.23
New South Wales 1 (NSW1)	18	17.46	1.46	0.19	0.22
New South Wales 2 (NSW2)	3	2.91	1.35	-	0.18

Number of individuals (N), mean number of individuals typed per locus (N_l), mean allelic richness (Ar), mean observed heterozygosity (H_o), and mean expected heterozygosity (H_e)

C. Pairwise F_{ST} among sampling regions derived from SNPs (left) and corresponding *P*-values (right; corrected using the Benjamini-Hochberg method).

	SA	cVIC1	cVIC2	eVIC	NSW1	NSW2
SA	-	0.028	0.032	0.035	0.056	0.667
cVIC1	0.214		0.032	0.011	0.011	0.545
cVIC2	0.218	0.017		0.011	0.011	0.270
eVIC	0.212	0.078	0.083		0.011	0.667
NSW1	0.247	0.121	0.127	0.089	-	1.00
NSW2	0.299	0.130	0.137	0.098	0.084	-

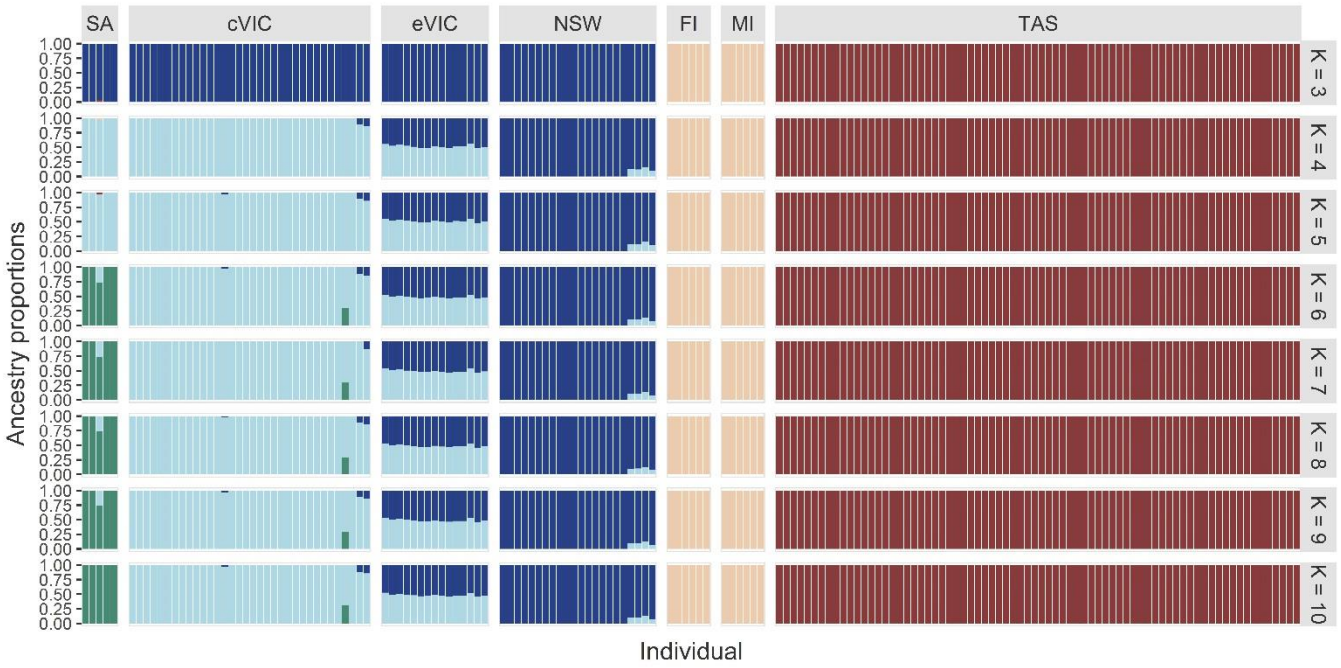
D. Hierarchical analysis of molecular variance (AMOVA) results.

Source of variation	Degrees of freedom	Sum of Squares	Sigma	Variation (%)	ϕ
Between regions	3	16162.64	104.7978	8.74	0.087
Between populations within regions	2	3905.875	36.7944	3.07	0.033
Between samples within populations	64	77554.75	154.6858	12.90	0.146
Within samples	70	63169.5	902.4214	75.28	0.247
Total	139	160792.8	1198.699	100	-

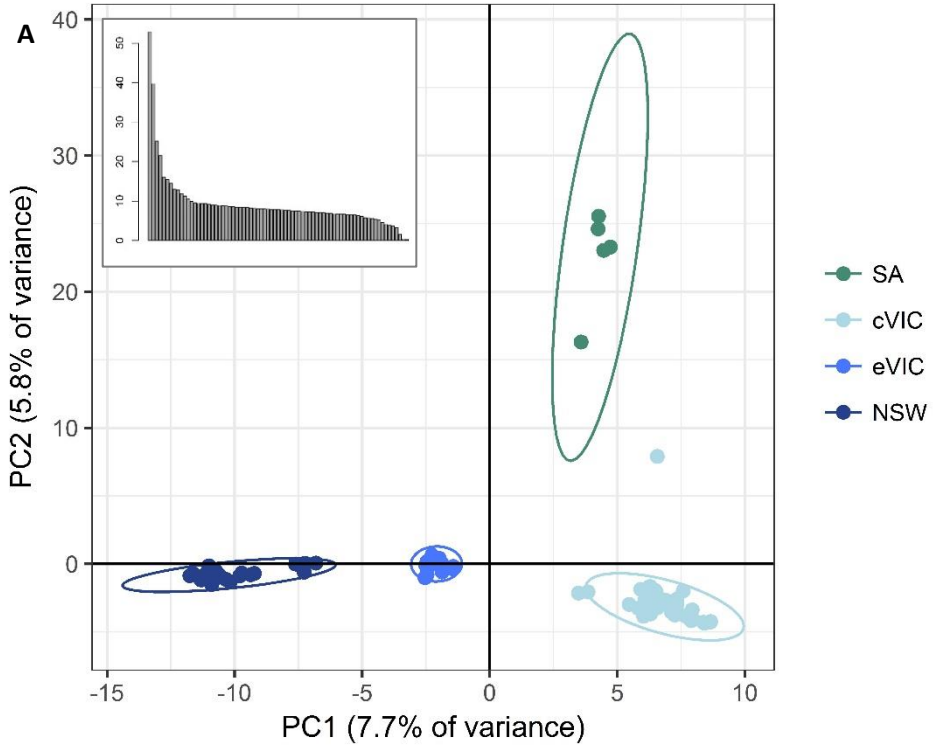
V. Results of fastSTRUCTURE for K=1−10 for different sampling regions when SNPs are filtered using HWE criteria. The K range was estimated by fastSTRUCTURE. Lowest marginal likelihood values are in bold.

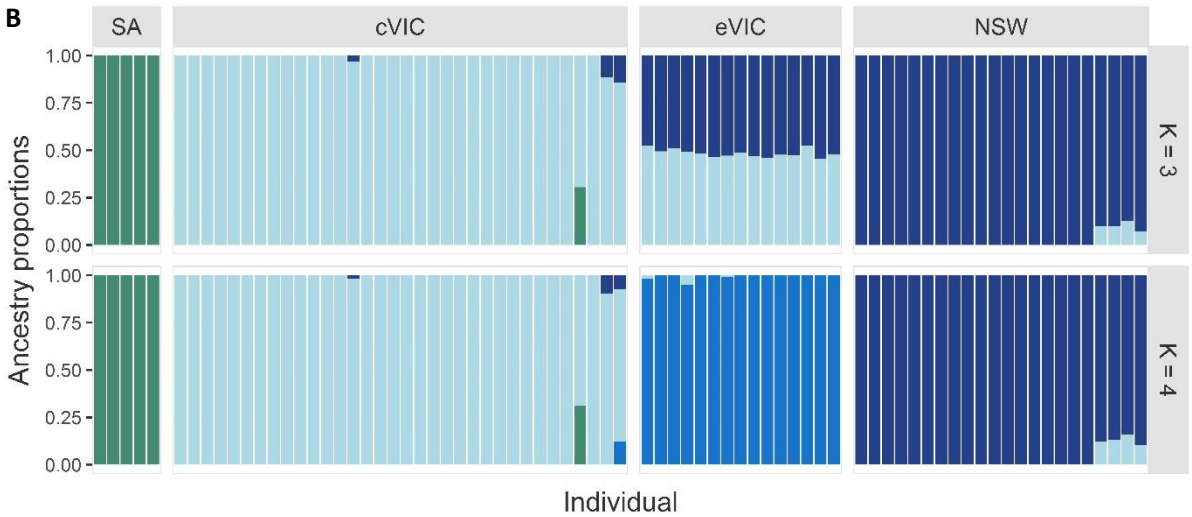
K	Sampling region			
	All	Mainland only	Tasmania only	Maria & Flinders
1	-0.896200	-0.803221	**-0.672007**	**-0.708640**
2	-0.750563	-0.795673	-0.672597	-0.709274
3	-0.735609	**-0.792636**	-0.688460	-0.709521
4	-0.731975	-0.799044	-0.703129	-0.709657
5	-0.732066	-0.803756	-0.699714	-0.709744
6	**-0.730519**	-0.799238	-0.703415	-0.709806
7	-0.730580	-0.807129	-0.691751	-0.709854
8	-0.730609	-0.806565	-0.691578	-0.709891
9	-0.730641	-0.807264	-0.682691	-0.709922
10	-0.730719	-0.800579	-0.673228	-0.709948
K based on model complexity	6	3	1	1
K based on model components	5	4	3	1

VI. fastSTRUCTURE assignment plots for K=3–10 including all individuals. Lack of distinction between some results with different K values reflects individuals being allocated a very small ancestry to a cluster (e.g., 0.00002).

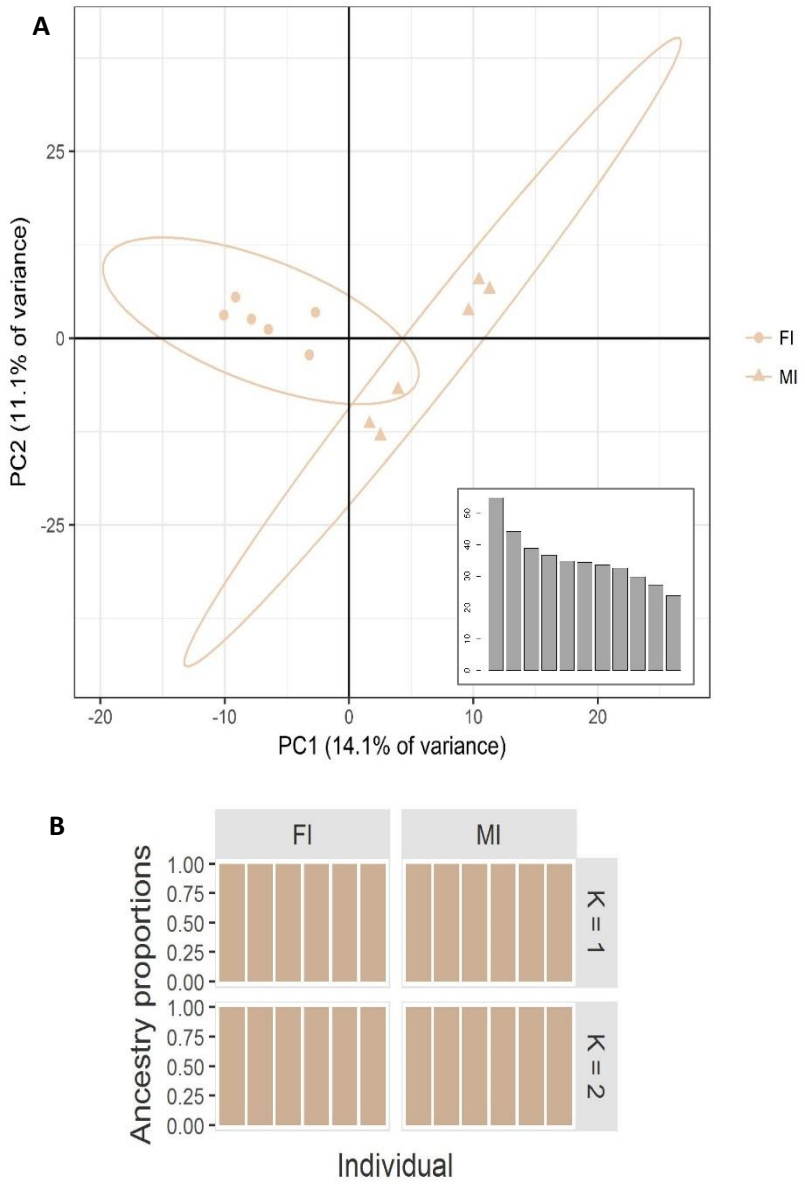


VII. The PCA (A) and fastSTRUCTURE (B) results for mainland individuals. Inset in the PCA graph is the corresponding eigenvalues, displayed as a bar plot. Each sampling region has a 99% confidence ellipse added.

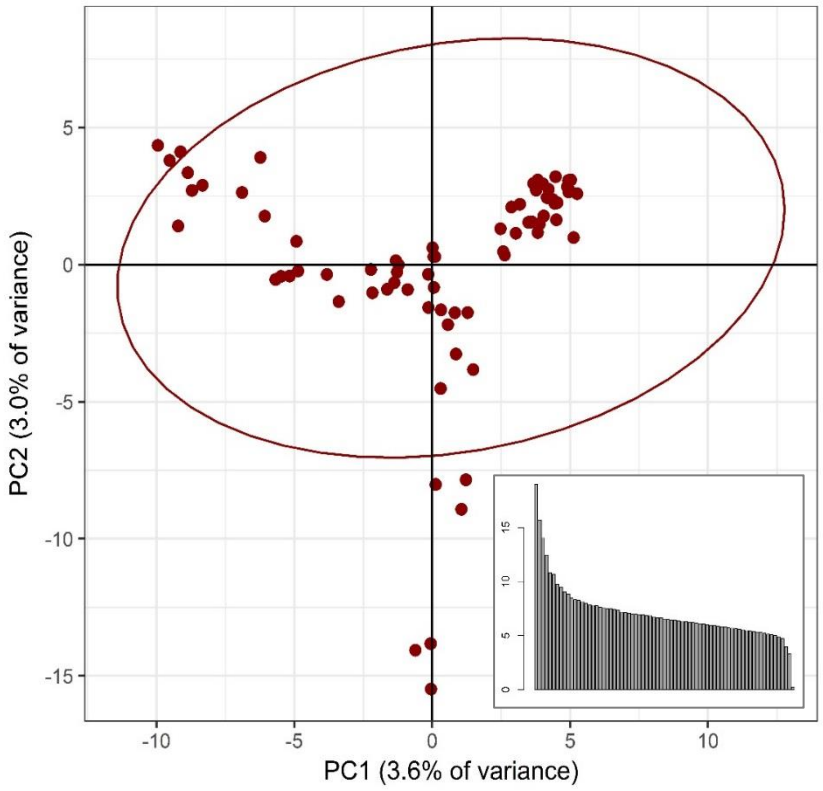




VIII. The PCA (A) and fastSTRUCTURE (B) results for island (Flinders and Maria) individuals. Inset in the PCA graph is the corresponding eigenvalues, displayed as a bar plot. Each sampling region has a 99% confidence ellipse added.



IX. The PCA results for Tasmanian individuals. Inset in the PCA graph is the corresponding eigenvalues, displayed as a bar plot. A 99% confidence ellipse is portrayed.



Chapter 6

Chapter 6.0 – General Discussion

6.1 Thesis overview

Sarcoptes scabiei is a pathogen of global concern, impacting humans, domestic animals, and wildlife (Arlian and Morgan 2017). Among hosts, *S. scabiei* has conserved pathology but varied prevalence and disease severity (Pence and Ueckermann 2002). Since its introduction to Australia, it has been documented in several native host species, with the bare-nosed wombat (*Vombatus ursinus*) experiencing the most severe morbidity (Skerratt 2005). Prior to this work, several studies have addressed the clinical effects of *S. scabiei* infection in wombats (Skerratt 2003b, a, Ruykys *et al.* 2013), but the mechanisms that result in host mortality were not well understood. Furthermore, only anecdotal reports existed regarding the impact of sarcoptic mange outbreaks on bare-nosed wombat populations and the success of treatment administration in free-living wombats. While bare-nosed wombats are of Least Concern, small and isolated populations likely exist throughout their range and may be at higher risk of extirpation due to disease and other threats (e.g., habitat loss). However, the population genetic structure had not been assessed across the *V. ursinus* range.

This thesis has combined empirical data, disease theory, and molecular techniques to address three main objectives. The first focused on the impact of sarcoptic mange at individual and population scales – specifically, behavioural and physiological effects of sarcoptic mange on individual bare-nosed wombats and the population level impact of disease outbreak events (Chapters 2 and 3). The second investigated the potential to control *S. scabiei* in wildlife populations, assessing the efficacy of current treatment protocols in eliminating *S. scabiei* when applied at a population scale during an outbreak event in the bare-nosed wombat host (Chapter 4). The third objective was to gain a better understanding of bare-nosed wombat ecology by exploring genetic structure across its range, among and within the three recognised subspecies (Chapter 5).

6.2 Summary of key results

Focusing on behaviour and physiology, I investigated the impact of *S. scabiei* infection on individual bare-nosed wombats. I concluded that (i) hair loss due to disease lead to an increase in amount of heat lost to the environment, (ii) mange disease resulted in an increased field metabolic rate, (iii) diseased animals spent less time foraging and more time resting, but that both activities were frequently interrupted, and (iv) diseased animals experienced changes in their fatty acid composition in adipose tissue consistent with inflammation. These findings suggest that mange disease manifests through a cascade of both behavioural and physiological changes which may result in compounding impacts on the host. Ultimately, mange disease incites an energetic burden that bare-nosed wombats cannot compensate for.

Using seven years of abundance data and four years of disease severity data, I assessed the impact and pattern of disease spread during a mange epizootic in a bare-nosed wombat population in northern Tasmania. I found that clinical signs of mange disease spread spatiotemporally from east to west through the population, resulting in a >94% decline in wombat abundance. These results reveal that outbreak events can lead to localised declines events under the right conditions, and likely extirpation events. These findings provide insights into identifying other at-risk populations. Further, understanding the pattern of disease spread may facilitate developing improved disease control regimes.

I undertook a population-scale disease control experiment using treatment protocols for mange disease developed for individual wombats. Using a combination of my empirical field data and a novel state-based model, I found that treating >80% of active burrows for 12 consecutive weeks was not sufficient to eradicate *S. scabiei* from Narawntapu National Park. Further, the state-based model revealed that treatment delivery success must have been low (~33%). Through sensitivity analyses I was able to demonstrate that improved treatment application methods and a longer lasting treatment may increase success of future disease control, thus guiding future applied research in this area.

Finally, I used genomic techniques to assess genetic structure and identify important populations across the *V. ursinus* range. Using >9,000 genome-wide single nucleotide polymorphisms, I detected three main genetic clusters associated with three geographically isolated regions – mainland Australia, Flinders and Maria Islands, and Tasmania – which was

consistent with current subspecific designations: *V. u. hirsutus* (mainland), *V. u. ursinus* (Bass Strait Island, Flinders), and *V. u. tasmaniensis* (Tasmania). I also identified a second population of the Vulnerable *V. u. ursinus* on Maria Island, resulting from a translocation event in the early 1970s. Last, I identified fragmentation as a major inhibitor to population connectivity in bare-nosed wombats.

6.3 Management implications

The research carried out in this thesis has direct implications for wombats and other host species impacted by *S. scabiei*. For wombats specifically (although not exclusively), mange pathology is highly conserved (Skerratt 2003b, Ruykys *et al.* 2013), and thus the physiological and behavioural impacts of *S. scabiei* I have shown in bare-nosed wombats may also be assumed in southern hairy-nosed wombats (*Lasiorhinus latifrons*). This suggests that both species may benefit from similar management actions to ameliorate the impacts of mange disease, such as appropriate use of treatments in the field. The third extant species of wombat, the Critically Endangered northern hairy-nosed wombat (*Lasiorhinus krefftii*), for which there are approximately 250 individuals, has yet to be impacted by mange. However, my findings suggest pathogen invasion into the two small, isolated, and genetically uniform northern hairy-nosed wombat populations may be possible and, thus, could pose a threat to this species. Efforts should continue to ensure that the *S. scabiei* mite is not introduced into the northern hairy-nosed wombat, as pathogen invasion could influence the trajectory of the species.

For the control and treatment of sarcoptic mange in wildlife populations, results presented here suggest that better treatment protocols should be developed. Anecdotal evidence has suggested that current treatment regimes are suitable for clearing an individual from *S. scabiei* infection. While my research does not dispute this, I did find logistical issues at the population scale (Chapter 4), suggesting that these methods are not yet suitable for use beyond a small number of individuals. Potential advances to the current protocol include employing a better application method with higher delivery success and utilizing a treatment that provides longer durations of protection to the host. The latter is probably more crucial in disease systems where the mite can persist in the environment (hosts that use dens or burrows). A potential candidate is Fluralaner, which has a higher retention time in the host system (up to 3 months)

(Kilp *et al.* 2016), and safety and efficacy trials will be required before using this drug on wombats. Additionally, other drug delivery methods in the field could be considered (e.g., topical, intravenous, ingestible). Topical application is the best approach for non-invasive delivery in this system, and developing treatment stations that can deliver multiple doses to increase the likelihood of successfully delivering one full treatment dose is worthy of consideration. Alternatively, more invasive, targeted treatment methods could be applied (catch-inject-release), but consideration would need to be given to their feasibility to employ at a population scale.

Findings from my population genomic analyses also have important management implications. The delineation of *V. ursinus* subspecies has direct implications for management of the species. The three subspecies are morphologically distinct, geographically isolated (no outbreeding among subspecies), and genetically differentiated, and thus separate management may be warranted. *Vombatus ursinus ursinus* is currently at highest relative risk given its limited range and isolated populations. There is potential to re-establish additional populations of *V. u. ursinus* on Bass Strait islands where they were formally extirpated, which would further secure the longevity of the subspecies. For *V. u. hirsutus* and *V. u. tasmaniensis*, which have larger and more continuous distributions, fragmentation and habitat loss pose the greatest risks. Populations isolated within these ranges, most notably the western distribution of *V. u. hirsutus*, may require management action, such as establishing corridors of connectivity or considering translocations.

6.4 Broader applications

The theoretical concepts developed in this thesis are not limited to wombats, but can be applied to other hosts impacted by sarcoptic mange disease, as well as other host-disease systems where the primary transmission pathway is environmental. Sarcoptic mange afflicts a wide range of abundant, vulnerable, and economically important wildlife and domestic animal species globally, including wolves (*Canis lupus*) (Jimenez *et al.* 2010), Spanish ibex (*Capra pyrenaica hispanica*) (León-Vizcaíno *et al.* 1999), black bear (*Ursus americanus*) (Fitzgerald *et al.* 2008), South American camelids (Bornstein 2010), and of course, humans (Alasaad *et al.* 2013). The relatively conserved pathology of *S. scabiei* infection across host species allows a

number of clinical findings to be generalised among species, and results of this research may provide valuable insight in ameliorating disease impact in other affected hosts. For example, wolves suffer similar heat loss due to sarcoptic mange infection (Cross *et al.* 2016), and may be afflicted by comparable shifts in their field metabolic rates. Additionally, the ideas developed here regarding *in-situ* control of *S. scabiei* have potential to be applied to other hosts that utilize dens (or burrows) through which the mite may persist off the host through time. For example, treatment methods here may be applied in red foxes (*Vulpes vulpes*), which use dens seasonally. The framework of employing a treatment regime that not only treats infected hosts, but also simultaneously provides protection to uninfected hosts while environmental reservoirs of pathogens deplete is not a new one. However, quantifying the importance of treatment duration and effective delivery – aspects of control that are often overlooked – provides new perspective for non-invasive control of other wildlife pathogens.

6.5 Future directions

The research presented in this thesis fills several knowledge gaps regarding the impacts and control of *S. scabiei* in wildlife populations and reveals novel disease and general ecological findings about the bare-nosed wombat. To improve capacity to predict and control *S. scabiei* outbreak events in wildlife, additional research is needed to understand factors that lead to epizootic events. The prevalence of *S. scabiei* often remains low in populations where the disease is endemic, and understanding what changes (e.g., environmental or host traits) lead to outbreak events will allow researchers to predict these events or create risk maps to manage disease outbreak.

Disease control efforts would also benefit from identifying transmission pathways in specific host systems. More research is needed to quantify the longevity of *S. scabiei* in the field environment. Further, there is a need to develop our understanding of infection dynamics in the field, including the necessary exposure rate of the host to mites and required mite densities for infection to establish. The role of density-dependence in *S. scabiei* transmission is not well understood and additional research into the effect of wombat burrow switching and sharing behaviour at different population densities would provide a novel understanding of transmission dynamics in this system.

Questions of general ecological interest remain regarding the bare-nosed wombat. Future research stemming from the work presented in this thesis may include investigation of divergence patterns and divergence date estimates among the recognized subspecies of *V. ursinus*. Further, estimation of subspecific population sizes and employment of population viability analyses would provide insight into at-risk groups and inform future management. With advances in molecular techniques and increasingly affordable molecular services, it would be of interest to sequence the genomes of the bare-nosed wombat, as well as the northern and southern hairy-nosed wombats.

Appendix

Appendix I – Burrows with resources have greater visitation and may enhance mange transmission among wombats

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I.I Abstract

Environmental exposure to *Sarcoptes scabiei* mites in burrows is considered the dominant mechanism of sarcoptic mange transmission among wombats. We document elevated activity of bare-nosed wombats at a burrow with subterranean water access relative to burrows without this resource, suggesting some burrows may contribute more to mange transmission than others.

Keywords: Parasitology, Vombatidae, disease, ectoparasite, behaviour, environment

I.II Introduction

Sarcoptic mange, caused by the microscopic burrowing mite, *Sarcoptes scabiei*, affects >100 mammalian species globally (Pence and Ueckermann 2002). Mange outbreaks in wildlife can result in population declines and extirpation events (León-Vizcaíno *et al.* 1999, Baker *et al.* 2000, Martin *et al.* 2018a), which are difficult to manage due to the combination of direct and indirect transmission pathways. *Sarcoptes scabiei* is known to persist off of the host in cool and humid environments for up to 19 days (10°C, 97% humidity) (Arlian and Morgan 2017). However, the role that indirect transmission plays is often not well understood, and likely depends on the suitability of the environmental reservoir for mite survival and the ability for the mite to come into contact with new hosts.

Bare-nosed wombats (*Vombatus ursinus*, also known as the common wombat) are highly impacted by *S. scabiei*, experiencing severe symptoms (parakeratosis, alopecia, emaciation), high mortality, and periodic epizootics causing population declines (Skerratt 2003b, Skerratt 2005, Martin *et al.* 2018a). It is widely considered that the stable, cool microclimate of the wombat burrow acts as an environmental reservoir for *S. scabiei*. Due to the solitary nature of bare-nosed wombats, transmission by direct interaction is likely limited, with the possible exception being when wombat densities are high and burrows are shared, though this is generally asynchronously (Skerratt *et al.* 2004a, Evans 2008). Transmission is thought to occur during exposure to mites deposited in the burrow by other wombats utilizing it at different times. Further, burrow sharing and visitation may increase due to burrow selectivity. For example, wombats prefer burrows near to optimal foraging areas (personal observation), and may show similar preferences to burrows containing other resources (e.g., water access). These selective behaviours may inadvertently increase the risk of both direct encounters and potential environmental transmission.

At Narawntapu National Park, an epizootic of sarcoptic mange depleted the wombat abundance by 75% from 2010 to 2015. Given the small population of wombats remaining in 2015, it was expected that burrow sharing would occur infrequently. However, it was hypothesized that preferred burrows may remain hotspots for activity. Here, we document activity rates of wombats at a burrow with a subterranean water source (‘water burrow’) in

peak dry season during a mange epizootic, to assess the impact of a limited resource on burrow sharing, with implications for disease transmission.

I.III Methods

A mange outbreak initiated in the bare-nosed wombat population at Narawntapu National Park (Tasmania, Australia, -41.1484, 146.6016) in 2010, spreading across the park causing a significant population decline (Martin *et al.* 2018a). In 2015, while the epizootic was ongoing, Scout Guard (SG560Z Zero Glow, 8m) surveillance cameras were deployed on 14 active wombat burrows (those with signs of wombat activity, e.g., presence of fresh diggings and scats) within a 50 ha area (Figure A.1). All cameras were active between October 28th and December 8th (late spring into summer), when the seasonally high water table had receded and water was a limiting resource within the park. The number of monitoring days ranged from 3−42 per burrow (Table A.1). Number of survey days varied due to several factors, including (i) varied battery life with media type, and (ii) compromised observations due to natural causes (i.e., obstruction to lens and field of view). Cameras were secured to wooden stakes or trees, approximately one meter off the ground and two meters from the burrow entrance, with the field of view encompassing the burrow opening. Each camera was programmed to take either one photograph (n=10) or video (10−15 seconds, n=4), with a 30 second delay between shots. Cameras recording video were placed on burrows with greatest activity. One burrow (TLC68) was categorized as a 'water burrow', or a burrow that contained a subterranean water source. This particular burrow had two tunnels visible from the entrance: one short tunnel (~1.5 m) that led to a water pool, and a second tunnel of unknown length. In addition to the visual confirmation of a water pool within, signs of drinking (e.g., wet snout and whiskers) were observed in wombats using this burrow: observations not made with other burrows.

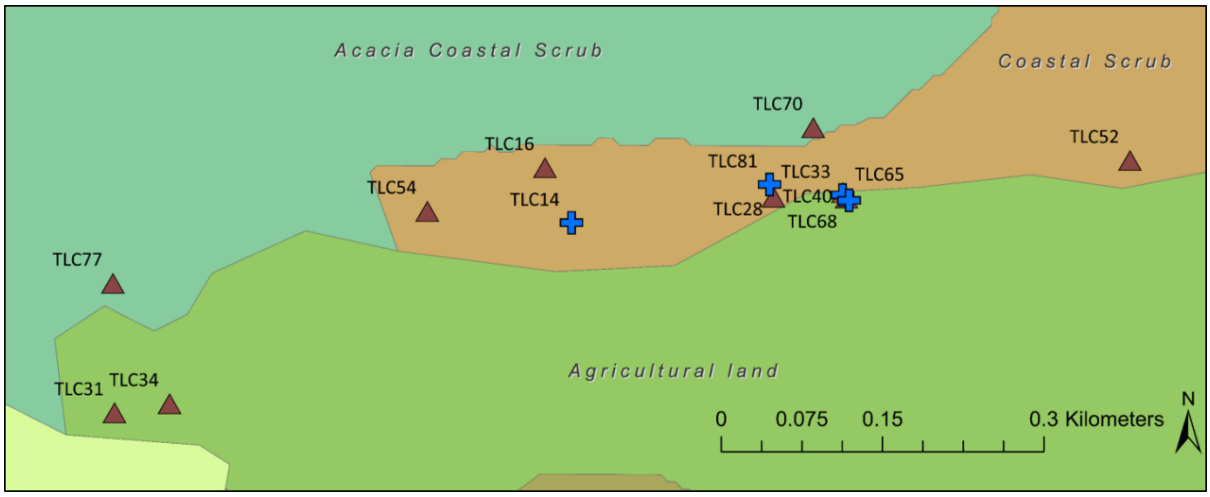

Figure A.1. Burrow locations and vegetation types in Narawntapu National Park. Burrows surveyed with video media are represented by crosses (blue) and still photo media are represented by triangles (brown).

Table A.1. Wombat activity (visits and entries) and mange presence (infected wombat visits) at fourteen burrows in Narawntapu National Park. The total number of instances a mange infected wombat visited (Mange infected (total)) and average number of visits by a mange-infected wombat across survey days (Mange infected (per day)) are shown. The water burrow is in bold, TLC68.

Camera	Media Type	Total days monitored	Wombat visits							Burrow entry						
			Total	Average visits (per day)	S.E.	Minimum visits (per day)	Maximum visits (per day)	Mange infected (total)	Mange infected (per day)	Total	Average entries (per day)	Mange infected (total)	Avg. Mange infected (per day)	Average duration (min)[a]	S.E.(min)	Max duration (min)
TLC16	Picture	17	1	0.06	0.01	0	1	0	0	-	-	-	-	-	-	-
TLC28	Picture	17	18	1.06	0.08	0	3	0	0	-	-	-	-	-	-	-
TLC31	Picture	42	6	0.14	0.01	0	1	0	0	-	-	-	-	-	-	-
TLC34	Picture	4	0	0	0	0	0	0	0	-	-	-	-	-	-	-
†TLC40	Picture	3	7	2.33	0.19	2	3	0	0	-	-	-	-	-	-	-
TLC52	Picture	13	18	1.38	0.09	0	3	0	0	-	-	-	-	-	-	-
TLC54	Picture	26	17	0.65	0.07	0	8	0	0	-	-	-	-	-	-	-
†TLC65	Picture	16	20	1.25	0.09	0	5	5	0.31	-	-	-	-	-	-	-
TLC70	Picture	25	19	0.76	0.03	0	2	4	0.16	-	-	-	-	-	-	-
TLC77	Picture	16	4	0.25	0.04	0	2	3	0.19	-	-	-	-	-	-	-
TLC14	Video	19	17	0.89	0.05	0	3	3	0.16	3	0.16	1	0.05	2.05	1.21	3.25
†TLC33	Video	16	23	1.44	0.1	0	6	1	0.06	3	0.19	1	0.06	1.75	n/a	1.75
TLC68	**Video**	**5**	**67**	**13.4**	**1.57**	**6**	**26**	**18**	**3.6**	**49**	**12.3**	**18**	**3.6**	**4.95**	**0.75**	**27.45**
TLC81	Video	19	14	0.74	0.06	0	4	3	0.16	5	0.26	1	0.05	2.9	0.8	4.4

[a]*Sample sizes for duration of time inside video monitored burrows: TLC14, n=2; TLC33, n=1, TLC68, n=41; TLC81, n=3*

†*Denotes burrows that were in very close proximity to the water burrow*

Images were processed (using program ExifPro), and wombat visits to each burrow were noted (Table A.1; Figure A.2). 'Visits' encompassed activity near the burrow entrance (within 0.5 m). Wombat visits ≥1 minute apart were considered independent for both media types. 'Entry' events were considered when the entire body of a wombat was inside the burrow. Burrow entry was only collected from cameras taking video footage (n=4, Table A.1), as it is more difficult to confirm entry from still photographs. Furthermore, for entry events where an individual wombat remained visible within the burrow (i.e., individual identity was unmistakable), the duration of the time spent inside the burrow was recorded. Mange was documented where possible (Simpson *et al.* 2016), however it is important to note that, in many cases, photographs did not capture sufficient body segments to definitively categorize a wombat as either healthy or mange infected. Thus, counts of wombats with mange were likely underestimates at all camera sites.

I.IV Results & Discussion

Cameras recording videos showed significantly more activity than those taking photographs (Welch two sample t-test: $P = 0.01$, t=-2.58, df=60.70), as expected based on the visual signs of activity near the selected burrows. Due to the bias in activity, the following analyses only incorporated the video monitored burrows (n=4, including the water burrow; Table A.1). The average number of visits per day was significantly higher at the water burrow (13.4 ± 1.57 S.E. visits per day) compared to the non-water burrows (1.0 ±0.07 S.E. visits per day; Welch two sample t-test: $P = 0.02$, t=-3.52, df=4.02). While wombats were not significantly more likely to enter the water burrow than non-water burrows, there was a trend (Welch two sample t-test: $P = 0.06$, t=-2.86, df= 3.03). When wombats did enter the water burrow, they spent more time inside (water burrow: 4.95 ± 0.75 S.E. minutes; non-water burrows:2.42 ±0.52 S.E. minutes; Welch two sample t-test: $P < 0.01$, t=-2.75, df= 30.66). There was a higher daily number of confirmed mange infected wombats entering the water burrow (n=18; Table A.1) than the other burrows (n=1 for each; Table A.1). On several occasions (n=5), ≥2 adult wombats were present at the water burrow. This was only observed in one occasion at a non-water burrow.

This study had some limitations, specifically the inability to consistently identify individual wombats. However, it is worth noting that water burrow activity rates were unlikely biased by

recurring visitation by a single wombat for two reasons. First, given the close proximity (<20m) of the water burrow to a non-water burrow (TLC33) in the same vegetation type and surveyed with the same media (video), we should expect similar activity rates at both burrows if this area was frequented for foraging, or if it was within a single wombat's core home range, however, this is not observed. Second, during the five survey days of the water burrow, we were able to identify at least 10 individual wombats based on hair loss patterns, size, the presence of an ear tag, or a combination of these (as opposed to between 3–4 individuals at the non-water, video media burrows). Thus, despite limitations in identifying individuals, this study offers critical insight to wombat behaviour during seasons with resource scarcity and has potential implications for disease spread in an otherwise solitary host.

To the best of our knowledge, we provide the first documentation of higher activity rates of both healthy and infected wombats at a burrow with a water resource, relative to burrows without signs of subterranean water access (e.g., water visible or wet soil at the burrow entrance) during a dry time of year. Water sources at NNP are scarce in summer months, and burrow sharing by wombats for access to this essential resource may facilitate mite transmission, directly, and potentially indirectly. Further research regarding the environmental transmission pathway for *S. scabiei* (e.g., environmental detection and survival experiments) would help inform conservation and management strategies for wombat populations experiencing mange outbreaks (e.g., prioritisation of burrows to target for treatment; Old *et al.* 2018).

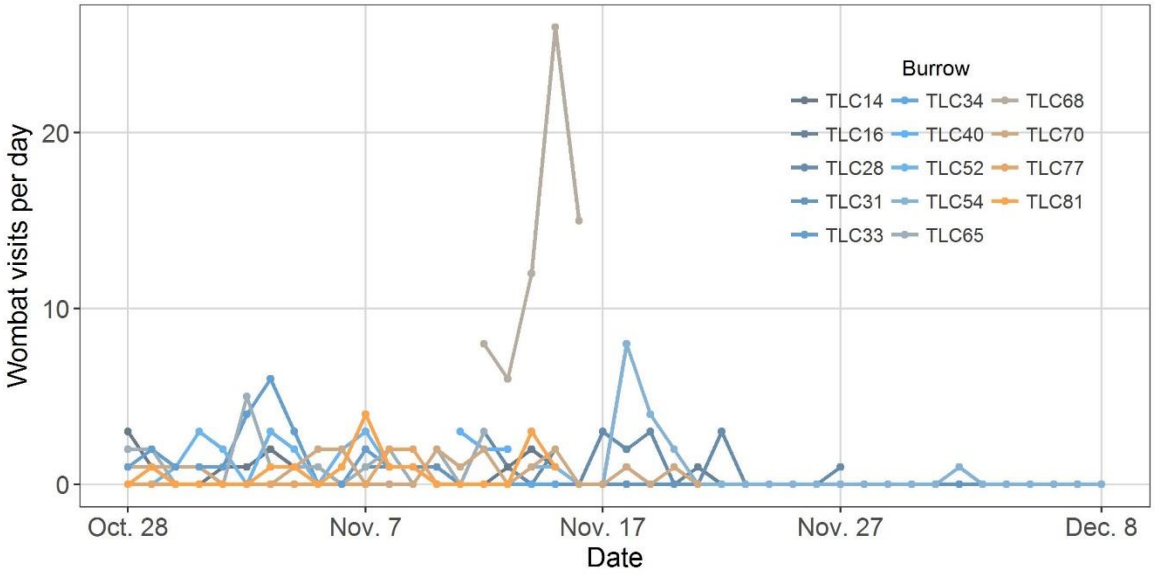

Figure A.2. The number of wombat visits to each burrow (per day) during the period from October 28th to December 8th 2015.

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